L1 ANSWER 1 OF 2 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2005:61554 CAPLUS
DOCUMENT NUMBER: 142:293602
TITLE: Extracellular RNA is a natural cofactor for the (auto-)activation of Factor VII-activating protease (FSAP)
AUTHOR(S): Nakazawa, Fumie; Kannemeier, Christian; Shibamiya, Aya; Song, Yutong; Tzima, Eleni; Schubert, Uwe; Koyama, Takatoshi; Niepmann, Michael; Trusheim, Heidi; Engelmann, Bernd; Preissner, Klaus T.
CORPORATE SOURCE: Graduate School of Allied Health Sciences, Tokyo Medical and Dental University, Bunkyo-ku, Tokyo, 113-8519, Japan
SOURCE: Biochemical Journal (2005), 385(3), 831-838
CODEN: BIJOAK; ISSN: 0264-6021
PUBLISHER: Portland Press Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB FSAP (Factor VII-activating protease) is a new plasma-derived serine protease with putative dual functions in hemostasis, including activation of coagulation Factor VII and generation of urinary-type plasminogen activator (urokinase). The (auto-)activation of FSAP is facilitated by polyanionic glycosaminoglycans, such as heparin or dextran sulfate, whereas calcium ions stabilize the active form of FSAP. In the present study, extracellular RNA was identified and characterized as a novel FSAP cofactor. The conditioned medium derived from various cell types such as smooth muscle cells, endothelial cells, osteosarcoma cells or CHO (Chinese-hamster ovary) cells contained an acidic factor that initiated (auto-)activation of FSAP. RNase A, but not other hydrolytic enzymes (proteases, glycanases and DNase), abolished the FSAP cofactor activity, which was subsequently isolated by anion-exchange chromatog. and unequivocally identified as RNA. In purified systems, as well as in plasma, different forms of natural RNA (rRNA, tRNA, viral RNA and artificial RNA) were able to (auto-)activate FSAP into the two-chain enzyme form. The specific binding of FSAP to RNA (but not to DNA) was shown by mobility-shift assays and UV crosslinking, thereby identifying FSAP as a new extracellular RNA-binding protein, the KD estimated to be 170-350 nM. Activation of FSAP occurred through an RNA-dependent template mechanism involving a nucleic acid size of at least 100 nt. In a purified system, natural RNA augmented the FSAP-dependent Factor VII activation several-fold (as shown by subsequent Factor Xa generation), as well as the FSAP-mediated generation of urokinase. The results provide evidence for the first time that extracellular RNA, present at sites of cell damage or vascular injury, can serve an important as yet unrecognized cofactor function in hemostasis by inducing (auto-)activation of FSAP through a novel surface-dependent mechanism.
REFERENCE COUNT: 21 THERE ARE 21 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L1 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1992:125378 CAPLUS
DOCUMENT NUMBER: 116:125378
TITLE: High molecular weight mucin-like glycoproteins of the bovine interphotoreceptor matrix
AUTHOR(S): Plantner, James J.
CORPORATE SOURCE: Sch. Med., Case West. Reserve Univ., Cleveland, OH, 44106, USA
SOURCE: Experimental Eye Research (1992), 54(1), 113-25
CODEN: EXERA6; ISSN: 0014-4835
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A very high-mol. weight mucin-like glycoprotein was isolated by gel

filtration of interphotoreceptor matrix (IPM) from fresh bovine eyes and purified to apparent homogeneity by CsCl/guanidine hydrochloride (GuHCl) equilibrium d. gradient centrifugation. Although a mol. weight in excess of 107 Da is suggested by gel filtration, the presence of SDS or GuHCl did not alter its elution position, indicating that the large size was not simply due to aggregation. Treatment of this material with disulfide reagents, however, led to a decrease in mol. size. On a relative basis, substantially more of this glycoprotein is present in IPM prepared from retina than from retinal pigment epithelium. While the carbohydrate and amino acid composition are not those of a true mucin, the large size and many other properties are quite mucin-like. The carbohydrate composition suggests the presence of both N- and O-glycosidically linked sugar chains. The presence of a mucin-type O-glycosidic linkage is indicated by its suceptibility to alkaline cleavage, with concomitant loss of serine and threonine and increase in 240 nm absorbance; production of a fluorescent product on reaction with cyanoacetamide; lectin-binding properties; and production of N-acetylgalactosaminitol on alkaline borohydride elimination. This glycoprotein was digested by pronase and trypsin, confirming its protein nature, but was resistant to digestion with chondroitin ABC lyase, hyaluronidase and heparinase, as well as RNAase, indicating that these components were not present to any appreciable extent. ELISA for cartilage keratan sulfate was also neg. Centrifugation in CsCl/GuHCl gradients indicated a d. much lower than that of a proteoglycan or nucleic acid as well. In vitro biosynthetic studies suggest that both retina and retinal pigment epithelium may be major sources of material in the IPM. The elution patterns of radioactivity were strikingly similar to the UV elution patterns of IPM. The medium from retinal incubations contained very high mol. weight material which was resistant to enzymes which hydrolyze glycosaminoglycans , suggesting that retina may be the source of this high-mol. weight, mucin-like glycoprotein.

L2 ANSWER 1 OF 2 CAPLUS COPYRIGHT 2006 ACS on STN
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DOCUMENT NUMBER: 142:293602
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AUTHOR(S): Nakazawa, Fumie; Kannemeier, Christian; Shibamiya, Aya; Song, Yutong; Tzima, Eleni; Schubert, Uwe; Koyama, Takatoshi; Niepmann, Michael; Trusheim, Heidi; Engelmann, Bernd; Preissner, Klaus T.
CORPORATE SOURCE: Graduate School of Allied Health Sciences, Tokyo Medical and Dental University, Bunkyo-ku, Tokyo, 113-8519, Japan
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L2 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2006 ACS on STN
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DOCUMENT NUMBER: 116:125378
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CODEN: EXERA6; ISSN: 0014-4835
DOCUMENT TYPE: Journal
LANGUAGE: English
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filtration of interphotoreceptor matrix (IPM) from fresh bovine eyes and purified to apparent homogeneity by CsCl/guanidine hydrochloride (GuHCl) equilibrium d. gradient centrifugation. Although a mol. weight in excess of 107 Da is suggested by gel filtration, the presence of SDS or GuHCl did not alter its elution position, indicating that the large size was not simply due to aggregation. Treatment of this material with disulfide reagents, however, led to a decrease in mol. size. On a relative basis, substantially more of this glycoprotein is present in IPM prepared from retina than from retinal pigment epithelium. While the carbohydrate and amino acid composition are not those of a true mucin, the large size and many other properties are quite mucin-like. The carbohydrate composition suggests the presence of both N- and O-glycosidically linked sugar chains. The presence of a mucin-type O-glycosidic linkage is indicated by its suceptibility to alkaline cleavage, with concomitant loss of serine and threonine and increase in 240 nm absorbance; production of a fluorescent product on reaction with cyanoacetamide; lectin-binding properties; and production of N-acetylgalactosaminitol on alkaline borohydride elimination. This glycoprotein was digested by pronase and trypsin, confirming its protein nature, but was resistant to digestion with chondroitin ABC lyase, hyaluronidase and heparinase, as well as RNAase, indicating that these components were not present to any appreciable extent. ELISA for cartilage keratan sulfate was also neg. Centrifugation in CsCl/GuHCl gradients indicated a d. much lower than that of a proteoglycan or nucleic acid as well. In vitro biosynthetic studies suggest that both retina and retinal pigment epithelium may be major sources of material in the IPM. The elution patterns of radioactivity were strikingly similar to the UV elution patterns of IPM. The medium from retinal incubations contained very high mol. weight material which was resistant to enzymes which hydrolyze glycosaminoglycans , suggesting that retina may be the source of this high-mol. weight, mucin-like glycoprotein.

L3 ANSWER 1 OF 2 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1975:439771 CAPLUS
DOCUMENT NUMBER: 83:39771
TITLE: Microspectrophotometric detection of heparin in mast cells and basophilic granulocytes stained metachromatically with Toluidine Blue O
AUTHOR(S): Tas, Johan; Geenen, Liesbeth H. M.
CORPORATE SOURCE: Histol. Lab., Univ. Amsterdam, Amsterdam, Neth.
SOURCE: Histochemical Journal (1975), 7(3), 231-48
CODEN: HISJAE; ISSN: 0018-2214
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A qual. microspectrometric detection method for heparin in situ was developed by using data obtained for a model system of polyacrylamide films containing pure glycosaminoglycans. This technique, based on the unique metachromatic properties of heparin with Toluidine Blue O in glycerol, was applied to rat peritoneal and mesenteric mast cells. After the smears containing the stained cells had been mounted in glycerol, a change with time of the recorded metachromatic peaks to lower wavelengths was found, leading to an equilibrium phase after some days. The metachromatic peaks recorded in this phase appeared to resemble closely the peak obtained for the heparin-Toluidine Blue O complex under similar conditions in the model expts. With rat mast cells it was found that nucleic acids, basic proteins, histamine, and lipids had no appreciable influence on the position of the final recorded peaks, nor did they influence the slope of the time course very much. This observed decrease with time in the wavelengths of the metachromatic peaks could be explained by the time necessary for equilibration of the cells in glycerol and by the possible influence of lower sulfated glycosaminoglycans on the peak of the heparin-Toluidine Blue O complex. It was found that the method could be used to detect unequivocally the presence of heparin in cells, even if they also contained 75% (mole/mole) of other, lower sulfated glycosaminoglycan. Only a limited number of cells was necessary with this method in contrast to biochem. detns. For the 1st time the presence of heparin in normal human basophilic granulocytes and mast cells was proved directly. The expts. indicated the occurrence of virtually similar sulfated heparins in human mast cells and basophilic granulocytes, as well as in pig mast cells. A higher sulfated heparin, however, might be present in rat mast cells.

L3 ANSWER 2 OF 2 MEDLINE on STN
ACCESSION NUMBER: 75151298 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1126855
TITLE: Microspectrophotometric detection of heparin in mast cells and basophilic granulocytes stained metachromatically with Toluidine Blue O.
AUTHOR: Tas J; Geenen L H
SOURCE: The Histochemical journal, (1975 May) Vol. 7, No. 3, pp. 231-48.
Journal code: 0163161. ISSN: 0018-2214.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 197508
ENTRY DATE: Entered STN: 10 Mar 1990
Last Updated on STN: 3 Feb 1997
Entered Medline: 8 Aug 1975
AB A qualitative microspectrophotometric detection method for heparin in situ has been developed, using data obtained previously with a model system of polyacrylamide films containing pure glycosaminoglycans (Tas, 1975). This technique, based on the unique metachromatic properties of

heparin with Toluidine Blue O in glycerol, has been worked out with rat peritoneal and mesenteric mast cells. After the smears containing the stained cells had been mounted in glycerol, a change with time of the recorded metachromatic peaks to lower wavelengths was found, leading to an equilibrium phase after some days. The metachromatic peaks recorded in this phase appeared to resemble closely the peak obtained for the heparin-Toluidine Blue O complex under similar conditions in the model experiments. With rat mast cells it was found that nucleic acids, basic proteins, histamine and lipids had no appreciable influence on the position of the final recorded peaks, nor did they influence the slope of the time course very much. This observed decrease with time in the wavelengths of the metachromatic peaks can be explained by the time necessary for equilibration of the cells in glycerol and by the possible influence of lower sulphated glycosaminoglycans on the peak of the heparin-Toluidine Blue O complex. It was found that the method can be used to detect unequivocally the presence of heparin in cells, even if they also contain up to 75% (mole/mole) of other, lower sulphated glycosaminoglycan. Only a limited number of cells is necessary with this method - in contrast to biochemical determinations. For the first time the presence of heparin in normal human basophilic granulocytes and mast cells has been proved directly. The experiments indicate the occurrence of virtually similar sulphated heparins in human mast cells and basophilic granulocytes, as well as in pig mast cells. A higher sulphated heparin, however, might be present in rat mast cells.

L4 ANSWER 1 OF 15 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2006:362835 CAPLUS
DOCUMENT NUMBER: 144:371890
TITLE: Manufacture of glycosaminoglycan fractions, removal of impurities form glycosaminoglycan fractions, and agents for them
INVENTOR(S): Koyama, Hiroshi; Imamura, Kimihiro; Koshinbo, Akira; Onuki, Yoji
PATENT ASSIGNEE(S): Seikagaku Kogyo Co., Ltd., Japan
SOURCE: Jpn. Kokai Tokkyo Koho, 12 pp.
CODEN: JKXXAF
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| JP 2006104461 | A2 | 20060420 | JP 2005-261689 | 20050909 |
| PRIORITY APPLN. INFO.: | | | JP 2004-264466 A | 20040910 |

AB The manufacturing method of glycosaminoglycan fractions with nucleic acid content (ethidium bromide method) <0.08 μg/mg, Fe content (atomic absorption measurement) ≤0.07 ppm, and protein content (Rowry method) ≤0.4% includes contacting the fraction solns. with activated charcoal, removing the activated charcoal, and isolating. Thus, preparing a fraction containing hyaluronic acid (I) from cock's combs, dissolving in purified water, adding activated charcoal treated in an autoclave, stirring for 1 h, and filtering gave a fraction showing nucleic acid content <0.08 μg/mg-I, Fe content 0.025-0.056 ppm, and protein content 0.004%.

L4 ANSWER 2 OF 15 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2005:61554 CAPLUS
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AUTHOR(S): Nakazawa, Fumie; Kannemeier, Christian; Shibamiya, Aya; Song, Yutong; Tzima, Eleni; Schubert, Uwe; Koyama, Takatoshi; Niepmann, Michael; Trusheim, Heidi; Engelmann, Bernd; Preissner, Klaus T.
CORPORATE SOURCE: Graduate School of Allied Health Sciences, Tokyo Medical and Dental University, Bunkyo-ku, Tokyo, 113-8519, Japan
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unequivocally identified as RNA. In purified systems, as well as in plasma, different forms of natural RNA (rRNA, tRNA, viral RNA and artificial RNA) were able to (auto-)activate FSAP into the two-chain enzyme form. The specific binding of FSAP to RNA (but not to DNA) was shown by mobility-shift assays and UV crosslinking, thereby identifying FSAP as a new extracellular RNA-binding protein, the KD estimated to be 170-350 nM. Activation of FSAP occurred through an RNA-dependent template mechanism involving a nucleic acid size of at least 100 nt. In a purified system, natural RNA augmented the FSAP-dependent Factor VII activation several-fold (as shown by subsequent Factor Xa generation), as well as the FSAP-mediated generation of urokinase. The results provide evidence for the first time that extracellular RNA, present at sites of cell damage or vascular injury, can serve an important as yet unrecognized cofactor function in hemostasis by inducing (auto-)activation of FSAP through a novel surface-dependent mechanism.

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L4 ANSWER 3 OF 15 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2004:885394 CAPLUS
DOCUMENT NUMBER: 142:133459
TITLE: Dried Rana catesbeiana extract having chondroitin sulfate
INVENTOR(S): Toida, Toshihiko; Han, Beom Su; Kim, Young Sik; Woo, Song Ji
PATENT ASSIGNEE(S): S. Korea
SOURCE: Repub. Korean Kongkae Taeho Kongbo, No pp. given
CODEN: KRXXA7
DOCUMENT TYPE: Patent
LANGUAGE: Korean
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| KR 2001017847 | A | 20010305 | KR 1999-33581 | 19990816 |

PRIORITY APPLN. INFO.: KR 1999-33581 19990816

AB Dried Rana catesbeiana extract and glycosaminoglycan segregated and purified from the dried extract are provided to effectively use the dried Rana catesbeiana extract for eye drops, rheumatism, new blood vessel growth control, osteoporosis, liver function improvement and climacterium by using the dried extract as an enzyme, protease. Dried Rana catesbeiana extract contains glycosaminoglycan having 6.0 of chondroitin, 26.9 of chondroitin sulfate C and 67.1 of chondroitin sulfate A. The manufacturing method of the dried Rana catesbeiana extract comprises the steps of: (1) dissolving the dried extract in buffer solution of pH 6.5-9.0 or NaOH solution of 0.05N; (2) adding protease like subtilisin in the mixture of the step (1); (3) treating the mixture of the step (2) with calcium chloride and 3-chloro-acetate to precipitate protein or nucleic acid; and (4) precipitating again the precipitate of the step (3) by ethanol and drying. The dried extract is dissolved in sodium chloride and precipitate complex is produced by adding quadrihydric ammonium. The precipitate complex is dissolved in a strong base and precipitated again by ethanol. Glycosaminoglycan is segregated for purifying from the dried Rana catesbeiana extract

L4 ANSWER 4 OF 15 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1993:250955 CAPLUS
DOCUMENT NUMBER: 118:250955
TITLE: Simultaneous preparation and quantitation of

proteoglycans by precipitation with Alcian blue
AUTHOR(S): Bjoernsson, Sven
CORPORATE SOURCE: Dep. Clin. Chem., Univ. Hosp., Lund, S-221 85, Swed.
SOURCE: Analytical Biochemistry (1993), 210(2), 282-91
CODEN: ANBCA2; ISSN: 0003-2697
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Conditions for specific interaction between Alcian blue and proteoglycans were optimized by comparing the differential spectra of Alcian blue obtained with purified chondroitin sulfate dissolved in water with the spectra obtained with nasal cartilage proteoglycans dissolved in synovial fluid. A method was then designed that provides specific precipitation of proteoglycans or glycosaminoglycans in 4M guanidine-HCl in the presence of protein, hyaluronic acid, or nucleic acids. The specificity is achieved by using a low pH in combination with detergent and high salt concentration Stepwise addition of reagents is necessary to avoid binding to Alcian blue to proteins and nucleic acids. All polyanions, except polysulfates, are first neutralized by lowering the pH to 1.5. By including detergent in this step, the hydrophobic protein regions are blocked and not accessible for binding with the dye. These regions could otherwise bind Alcian blue by hydrophobic interaction. When the Alcian blue reagent is added after, only the polysulfated mols. will remain charged and free to interact with Alcian blue. At least 0.4M guanidine-HCl is required to abolish the neg. interference by proteins. All sulfated glycosaminoglycans are precipitated at 0.4M guanidine-HCl. With increasing guanidine-HCl concns., the different glycosaminoglycans are precipitated in accordance with the critical electrolyte concentration of the resp. glycosaminoglycan. The Alcian blue precipitation can be performed at different concns. of guanidine-HCl in order to sep. different classes of proteoglycans. Excess dye and contaminating proteins are removed by a wash in a DMSO-MgCl2 solution and the precipitate is dissolved in a mixture of guanidine-HCl and propanol. For quantitation, the absorbance is recorded in a microplate reader with the 600-nm filter, the assay being linear between 0.5 and 20 μg proteoglycan. Since no digestion of samples with protease is needed, the proteoglycans are recovered in native form. The proteoglycan-Alcian blue complexes dissociate in the guanidine-HCl/propanol mixture and the proteoglycans can be selectively precipitated with propanol. The dye is used for quantitation and the proteoglycans can be utilized for further anal.

L4 ANSWER 5 OF 15 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1993:250127 CAPLUS
DOCUMENT NUMBER: 118:250127
TITLE: Purification and characterization of monkey (Macaca nemestrina) tracheobronchial mucin
AUTHOR(S): Devaraj, Halagowder; Griffith, James W.; Sheykhnazari, Mostafa; Naziruddin, Bashoo; Sachdev, Goverdhan P.; Bhavanandan, V. P.
CORPORATE SOURCE: Coll. Pharm., Univ. Oklahoma, Oklahoma City, OK, USA
SOURCE: Archives of Biochemistry and Biophysics (1993), 302(1), 285-93
CODEN: ABBIA4; ISSN: 0003-9861
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A major mucin glycoprotein was purified from monkey (M. nemestrina) bronchoalveolar lavages by gel filtration, delipidation, and a series of d. gradient centrifugations in cesium trifluoroacetate/guanidinium chloride. Lipids noncovalently associated with the mucin amounted to 24-36% by weight and consisted primarily of phospholipds and glycolipids. The mucin preparation was free of low-mol.-weight

protein/glycoprotein contaminants, glycosaminoglycans /proteoglycans, and nucleic acids. The weight-average mol. weight and radius of gyration of the mucin in buffer containing 6M guanidinium chloride was estimated to be .apprx.1.56 x 106 and 100 nm, resp., by laser light scattering technique. When the mucin was dissolved in 0.15M NaCl, a considerably higher mol. weight of .apprx.5.05 x 106 and a larger radius of gyration of .apprx.127 nm were observed suggesting aggregation of the mucin mols. Amino acid composition of the glycoprotein was characteristic of mucins with threonine, serine, glutamic acid, proline, glycine, and alanine comprising 63%. The total carbohydrate content was 71.5% and consisted of GalNAc, GlcNAc, Gal, sialic acids, and fucose in the molar ratio of 1.0:2.2:2.4:1.4:1.4:1.2 with no detectable mannose. Alkaline borohydride treatment indicated that 65% of the threonine and 27% of the serine are substituted by saccharides via GalNAc residues. An antisera produced against the purified mucin was found to react well with the native and weakly with the deglycosylated mucins and will be useful for immunoassays. A second, minor, mucin glycoprotein obtained during the purifn. was also partially characterized.

L4 ANSWER 6 OF 15 CAPLUS COPYRIGHT 2006 ACS on STN
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DOCUMENT NUMBER: 116:125378
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CODEN: EXERA6; ISSN: 0014-4835
DOCUMENT TYPE: Journal
LANGUAGE: English
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glycoprotein.

L4 ANSWER 7 OF 15 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1988:91226 CAPLUS
DOCUMENT NUMBER: 108:91226
TITLE: Densitometric assay of nanogram quantities of proteoglycans precipitated on nitrocellulose membrane with safranin O
AUTHOR(S): Lammi, Mikko; Tammi, Markku
CORPORATE SOURCE: Dep. Anat., Univ. Kuopio, Kuopio, SF-70211, Finland
SOURCE: Analytical Biochemistry (1988), 168(2), 352-7
CODEN: ANBCA2; ISSN: 0003-2697
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Proteoglycan (PG) and glycosaminoglycan (GAG) samples corresponding to a min. of 10 ng uronic acid were reliably quantified as ppts. with the cationic dye Safranin O, collected by vacuum-aided filtration onto a cellulose acetate/nitrate membrane in a standard 96-well dot assay apparatus The reflectances of the precipitation dots were measured by automatic densitometric scanning of the membrane sheets. Standard GAGs produced reflectance values which were related to the number of anionic groups per unit disaccharide; hyaluronate and keratan sulfate gave lower values while heparin yielded values higher than those of chondroitin sulfates. The presence of 8M urea, 1% Triton X 100, 30% sucrose, 0.02% NaN3, or mixts. of proteinase inhibitors and various buffers did not markedly influence the reflectances, while 4M guanidinium chloride and 3M CsCl reduced the sensitivity of the assay to 30-50 ng. Samples containing SDS were not applicable because SDS precipitated with Safranin O. Proteins showed virtually no response, while nucleic acids gave significant although smaller reflectances than GAGs. Owing to its marked sensitivity and convenience, the method is particularly suitable for the detection of PGs during their preparative purifn. and fractionation as well as in various anal. assays.

L4 ANSWER 8 OF 15 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1986:605107 CAPLUS
DOCUMENT NUMBER: 105:205107
TITLE: Renal plasma membrane receptors for certain modified serum albumins. Evidence for participation of a heparin receptor
AUTHOR(S): Ranganathan, Perungavur N.; Mego, John L.
CORPORATE SOURCE: Biol. Dep., Univ. Alabama, University, AL, 35486, USA
SOURCE: Biochemical Journal (1986), 239(3), 537-43
CODEN: BIJOAK; ISSN: 0306-3275
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The binding of formaldehyde-treated (f-alb), reduced-carboxymethylated (ac-alb) or reduced-acetamidated (am-alb) bovine serum albumins to purified rat renal plasma membranes was studied. Radioiodinated f-alb or ac-alb bound to kidney membranes, whereas am-alb neither bound significantly nor competed with f-alb binding to kidney membranes. The binding was specific, saturable, and heat- and proteinase -sensitive. Competition studies showed that f-alb and ac-alb sites may be the same on these membranes. To determine the role played by charge in binding, competition expts. with polyanions were performed. Polyanions, such as nucleic acid or glycosaminoglycans, were effective competitors of f-alb binding to cell membranes. Heparin was especially inhibitory, being several-fold more so than chondroitin sulfate. Completely reduced and carboxymethylated albumin was a better competitor than its partially modified counterpart. Furthermore, f-alb was a significant competitor of [35S]heparin binding to kidney membranes. Also, partially purified heparin receptor demonstrated specific binding of 125I-f-alb. The data suggested that a heparin receptor is

responsible for binding and internalization of i.v. injected f-alb. A Scatchard plot revealed 2 classes of receptors with dissociation consts. of 3.2 + 10-6 and 4.7 + 10-5 M.

L4 ANSWER 9 OF 15 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1985:558422 CAPLUS
DOCUMENT NUMBER: 103:158422
TITLE: Basic protein-glycosaminoglycan interaction as a possible primary cause of vascular and cardiac damage
AUTHOR(S): Tedeschi, Guido G.; Cingolani, Rosalia Tacconi
CORPORATE SOURCE: Sch. Clin. Chem. Bacteriol., Univ. Camerino, Camerino, 62032, Italy
SOURCE: IRCS Medical Science (1985), 13(6), 514
CODEN: IMSCE2; ISSN: 0268-8220
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The addition of hyaluronic acid and/or chondroitin sulfate to histone or human globin solns. caused the immediate formation of voluminous aggregates of eosinophil particles and fibrouis material with strong affinity towards Alcian blue, insol. in the pH range 2-13. The addition of hyaluronic acid and/or chondroitin sulfate solns. to β-hemolytic Streptococcus group A cultures from the beginning of the incubation or at various time intervals, caused rapid agglutination and sedimentation, lysis, and the appearance of granular material which condensed in large aggregates held together by fibrous structures embedding the bacterial bodies. A similar addition to the 4500 + g supernatant gave rise to a precipitate containing a small number of altered bacteria and a relatively large amount of low mol. weight nucleic acids. No nucleic acids in a detectable amount were present in the precipitate produced following addition of hyaluronic acid and/or chondroitin sulfate to the 0.45 μm filtrate of the cultures. The expts. where saline solns. of purified histone proteins and glycosaminoglycans were used indicate that the precipitation may be attributed to the presence of basic proteins within the cultures. The initial formation of particulate material in vivo accompanied by deposit at the cardiac and vascular level, could be attributed to interaction between basic proteins and glycosaminoglycans, independent of the presence of lipid components. This process could be enhanced during the course of inflammation, especially if it was caused or accompanied by bacterial growth.

L4 ANSWER 10 OF 15 MEDLINE on STN
ACCESSION NUMBER: 2005028212 MEDLINE
DOCUMENT NUMBER: PubMed ID: 15654766
TITLE: Extracellular RNA is a natural cofactor for the (auto-)activation of Factor VII-activating protease (FSAP).
AUTHOR: Nakazawa Fumie; Kannemeier Christian; Shibamiya Aya; Song Yutong; Tzima Eleni; Schubert Uwe; Koyama Takatoshi; Niepmann Michael; Trusheim Heidi; Engelmann Bernd; Preissner Klaus T
CORPORATE SOURCE: Graduate School of Allied Health Sciences, Tokyo Medical and Dental University, Bunkyo-ku, Tokyo 113-8519, Japan.
SOURCE: The Biochemical journal, (2005 Feb 1) Vol. 385, No. Pt 3, pp. 831-8.
Journal code: 2984726R. E-ISSN: 1470-8728.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200507
ENTRY DATE: Entered STN: 19 Jan 2005
Last Updated on STN: 31 Jul 2005
Entered Medline: 29 Jul 2005

AB FSAP (Factor VII-activating protease) is a new plasma-derived serine protease with putative dual functions in haemostasis, including activation of coagulation Factor VII and generation of urinary-type plasminogen activator (urokinase). The (auto-)activation of FSAP is facilitated by polyanionic glycosaminoglycans, such as heparin or dextran sulphate, whereas calcium ions stabilize the active form of FSAP. In the present study, extracellular RNA was identified and characterized as a novel FSAP cofactor. The conditioned medium derived from various cell types such as smooth muscle cells, endothelial cells, osteosarcoma cells or CHO (Chinese-hamster ovary) cells contained an acidic factor that initiated (auto-)activation of FSAP. RNase A, but not other hydrolytic enzymes (proteases, glycanases and DNase), abolished the FSAP cofactor activity, which was subsequently isolated by anion-exchange chromatography and unequivocally identified as RNA. In purified systems, as well as in plasma, different forms of natural RNA (rRNA, tRNA, viral RNA and artificial RNA) were able to (auto-)activate FSAP into the two-chain enzyme form. The specific binding of FSAP to RNA (but not to DNA) was shown by mobility-shift assays and UV crosslinking, thereby identifying FSAP as a new extracellular RNA-binding protein, the K(D) estimated to be 170-350 nM. Activation of FSAP occurred through an RNA-dependent template mechanism involving a nucleic acid size of at least 100 nt. In a purified system, natural RNA augmented the FSAP-dependent Factor VII activation several-fold (as shown by subsequent Factor Xa generation), as well as the FSAP-mediated generation of urokinase. Our results provide evidence for the first time that extracellular RNA, present at sites of cell damage or vascular injury, can serve an important as yet unrecognized cofactor function in haemostasis by inducing (auto-)activation of FSAP through a novel surface-dependent mechanism.

L4 ANSWER 11 OF 15 MEDLINE on STN
ACCESSION NUMBER: 93289951 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8512063
TITLE: Simultaneous preparation and quantitation of proteoglycans by precipitation with alcian blue.
AUTHOR: Bjornsson S
CORPORATE SOURCE: Department of Clinical Chemistry, University Hospital, Lund, Sweden.
SOURCE: Analytical biochemistry, (1993 May 1) Vol. 210, No. 2, pp. 282-91.
Journal code: 0370535. ISSN: 0003-2697.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199307
ENTRY DATE: Entered STN: 23 Jul 1993
Last Updated on STN: 6 Feb 1998
Entered Medline: 9 Jul 1993

AB Conditions for specific interaction between Alcian blue and proteoglycans were optimized by comparing the differential spectra of Alcian blue obtained with purified chondroitin sulfate dissolved in water with the spectra obtained with nasal cartilage proteoglycans dissolved in synovial fluid. A method was then designed that provides specific precipitation of proteoglycans or glycosaminoglycans in 4 M guanidine - HCl in the presence of protein, hyaluronic acid, or nucleic acids. The specificity is achieved by using a low pH in combination with detergent and high salt concentration. Stepwise addition of reagents is necessary to avoid binding of Alcian blue to proteins and nucleic acids. All polyanions, except polysulfates, are first neutralized by lowering the pH to 1.5. By including detergent in this step, the hydrophobic protein regions are blocked and not accessible for binding with the dye. These regions could otherwise bind Alcian blue by hydrophobic

interaction. When the Alcian blue reagent is added after, only the polysulfated molecules will remain charged and free to interact with Alcian blue. At least 0.4 M guanidine-HCl is required to abolish the negative interference by proteins. All sulfated glycosaminoglycans are precipitated at 0.4 M guanidine-HCl. With increasing guanidine-HCl concentrations, the different glycosaminoglycans are precipitated in accordance with the critical electrolyte concentration of the respective glycosaminoglycan. The Alcian blue precipitation can be performed at different concentrations of guanidine-HCl in order to separate different classes of proteoglycans. Excess dye and contaminating proteins are removed by a wash in a DMSO-MgCl2 solution and the precipitate is dissolved in a mixture of guanidine-HCl and propanol. For quantitation, the absorbance is recorded in a microplate reader with the 600-nm filter, the assay being linear between 0.5 and 20 micrograms proteoglycan. Since no digestion of samples with protease is needed, the proteoglycans are recovered in native form. The proteoglycan-Alcian blue complexes dissociate in the guanidine-HCl/propanol mixture and the proteoglycans can be selectively precipitated with propanol. The dye is used for quantitation and the proteoglycans can be utilized for further analysis.

L4 ANSWER 12 OF 15 MEDLINE on STN
ACCESSION NUMBER: 93228357 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8470904
TITLE: Purification and characterization of monkey (Macaca nemestrina) tracheobronchial mucin.
AUTHOR: Devaraj H; Griffith J W; Sheykhnazari M; Naziruddin B; Sachdev G P; Bhavanandan V P
CORPORATE SOURCE: Department of Biological Chemistry, M. S. Hershey Medical Center, Pennsylvania State University, Hershey 17033.
CONTRACT NUMBER: HL42651 (NHLBI)
SOURCE: Archives of biochemistry and biophysics, (1993 Apr) Vol. 302, No. 1, pp. 285-93.
Journal code: 0372430. ISSN: 0003-9861.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199305
ENTRY DATE: Entered STN: 21 May 1993
Last Updated on STN: 21 May 1993
Entered Medline: 12 May 1993

AB A major mucin glycoprotein was purified from monkey (Macaca nemestrina) bronchoalveolar lavages by gel filtration, delipidation, and a series of density gradient centrifugations in cesium trifluoroacetate/guanidinium chloride. Lipids noncovalently associated with the mucin amounted to 24-36% by weight and consisted primarily of phospholipids and glycolipids. The mucin preparation was free of low-molecular-weight protein/glycoprotein contaminants, glycosaminoglycans/proteoglycans, and nucleic acids. The weight-average molecular weight and radius of gyration of the mucin in buffer containing 6 M guanidinium chloride was estimated to be approximately 1.56 x 10(6) and 100 nm, respectively, by laser light scattering technique. When the mucin was dissolved in 0.15 M NaCl, a considerably higher molecular weight of approximately 5.05 x 10(6) and a larger radius of gyration of approximately 127 nm were observed suggesting aggregation of the mucin molecules. Amino acid composition of the glycoprotein was characteristic of mucins with threonine, serine, glutamic acid, proline, glycine, and alanine comprising 63%. The total carbohydrate content was 71.5% and consisted of GalNAc, GlcNAc, Gal, sialic acids, and fucose in the molar ratio of 1.0:2.2:2.4:1.4:1.2 with no detectable mannose. Alkaline borohydride treatment indicated that 65% of the threonine and 27% of the serine are substituted by saccharides via

GalNAc residues. An antisera produced against the purified mucin was found to react well with the native and weakly with the deglycosylated mucins and will be useful for immunoassays. A second, minor, mucin glycoprotein obtained during the purification was also partially characterized.

L4 ANSWER 13 OF 15 MEDLINE on STN
ACCESSION NUMBER: 92175078 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1541329
TITLE: High molecular weight mucin-like glycoproteins of the bovine interphotoreceptor matrix.
AUTHOR: Plantner J J
CORPORATE SOURCE: Lorand V. Johnson Laboratory for Research in Ophthalmology, Department of Surgery, Case Western Reserve University, Cleveland, OH 44106.
CONTRACT NUMBER: EY 06571 (NEI)
SOURCE: Experimental eye research, (1992 Jan) Vol. 54, No. 1, pp. 113-25.
Journal code: 0370707. ISSN: 0014-4835.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199204
ENTRY DATE: Entered STN: 24 Apr 1992
Last Updated on STN: 24 Apr 1992
Entered Medline: 8 Apr 1992

AB A very high molecular weight mucin-like glycoprotein was isolated by gel filtration of interphotoreceptor matrix (IPM) from fresh bovine eyes and purified to apparent homogeneity by cesium chloride/guanidine hydrochloride (GuHCl) equilibrium density gradient centrifugation. Although a molecular weight in excess of 10(7) Da is suggested by gel filtration, the presence of SDS or GuHCl did not alter its elution position, indicating that the large size was not simply due to aggregation. Treatment of this material with disulfide reagents, however, led to a decrease in molecular size. On a relative basis, substantially more of this glycoprotein is present in IPM prepared from retina than from retinal pigment epithelium. While the carbohydrate and amino acid composition are not those of a true 'mucin', the large size and many other properties are quite 'mucin-like'. The carbohydrate composition suggests the presence of both N- and O-glycosidically linked sugar chains. The presence of a mucin-type O-glycosidic linkage is indicated by its susceptibility to alkaline cleavage, with concomitant loss of serine and threonine and increase in 240 nm absorbance; production of a fluorescent product upon reaction with cyanoacetamide; lectin binding properties; and production of N-acetylgalactosaminitol upon alkaline borohydride elimination. This glycoprotein was digested by pronase and trypsin, confirming its protein nature, but was resistant to digestion with chondroitin ABC lyase, hyaluronidase and heparinase, as well as RNAase, indicating that these components were not present to any appreciable extent. ELISA for cartilage keratan sulfate was also negative. Centrifugation in CsCl/GuHCl gradients indicated a density much lower than that of a proteoglycan or nucleic acid as well. In vitro biosynthetic studies suggest that both retina and retinal pigment epithelium may be major sources of material in the IPM. The elution patterns of radioactivity were strikingly similar to the UV elution patterns of IPM. The medium from retinal incubations contained very high molecular weight material which was resistant to enzymes which hydrolyse glycosaminoglycans, suggesting that retina may be the source of this high molecular weight, mucin-like glycoprotein.

L4 ANSWER 14 OF 15 MEDLINE on STN
ACCESSION NUMBER: 88207935 MEDLINE
DOCUMENT NUMBER: PubMed ID: 3129962

TITLE: Densitometric assay of nanogram quantities of proteoglycans precipitated on nitrocellulose membrane with Safranin O.
AUTHOR: Lammi M; Tammi M
CORPORATE SOURCE: Department of Anatomy, University of Kuopio, Finland.
SOURCE: Analytical biochemistry, (1988 Feb 1) Vol. 168, No. 2, pp. 352-7.
Journal code: 0370535. ISSN: 0003-2697.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198805
ENTRY DATE: Entered STN: 8 Mar 1990
Last Updated on STN: 8 Mar 1990
Entered Medline: 31 May 1988

AB Proteoglycan (PG) and glycosaminoglycan (GAG) samples corresponding to a minimum of 10 ng of uronic acid were reliably quantified as precipitates with the cationic dye Safranin O, collected by vacuum-aided filtration onto a cellulose acetate/nitrate membrane in a standard 96-well dot assay apparatus. The reflectances of the precipitation dots were measured by automatic densitometric scanning of the membrane sheets. Standard GAGs produced reflectance values which were related to the number of anionic groups per unit disaccharide; hyaluronate and keratan sulfate gave lower values while heparin yielded values higher than those of chondroitin sulfates. The presence of 8 M urea, 1% Triton X-100, 30% sucrose, 0.02% NaN3, or mixtures of proteinase inhibitors and various buffers did not markedly influence the reflectances, while 4 M guanidinium chloride and 3 M CsCl reduced the sensitivity of the assay to 30-50 ng. Samples containing sodium dodecyl sulfate (SDS) were not applicable because SDS precipitated with Safranin O. Proteins showed virtually no response, while nucleic acids gave significant although smaller reflectances than GAGs. Owing to its marked sensitivity and convenience the method is particularly suitable for the detection of PGs during their preparative purification and fractionation as well as in various analytical assays.

L4 ANSWER 15 OF 15 MEDLINE on STN
ACCESSION NUMBER: 87156514 MEDLINE
DOCUMENT NUMBER: PubMed ID: 3030264
TITLE: Renal plasma membrane receptors for certain modified serum albumins. Evidence for participation of a heparin receptor.
AUTHOR: Ranganathan P N; Mego J L
SOURCE: The Biochemical journal, (1986 Nov 1) Vol. 239, No. 3, pp. 537-43.
Journal code: 2984726R. ISSN: 0264-6021.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198704
ENTRY DATE: Entered STN: 3 Mar 1990
Last Updated on STN: 3 Mar 1990
Entered Medline: 1 Apr 1987

AB Binding of formaldehyde-treated (f-alb), reduced-carboxymethylated (ac-alb) or reduced-acetamidated (am-alb) bovine serum albumins to purified rat renal plasma membranes was studied. Radioiodinated f-alb or ac-alb bound to kidney membranes while am-alb neither bound significantly nor competed with f-alb binding to kidney membranes. The binding was specific, saturable and heat- and proteinase -sensitive. Competition studies showed that f-alb and ac-alb sites may be the same on these membranes. To determine the role played by charge in binding, competition experiments with polyanions were performed. Polyanions such as nucleic acid or

glycosaminoglycans were effective competitors of f-alb binding to cell membranes. Heparin was especially inhibitory, being several-fold more so than chondroitin sulphate. Completely reduced and carboxymethylated albumin was a better competitor than its partially modified counterpart. Furthermore, f-alb was a significant competitor of [35S]heparin binding to kidney membranes. Also, partially purified heparin receptor demonstrated specific binding of 125I-f-alb. These data suggest that a heparin receptor is responsible for binding and internalization of intravenously injected f-alb. A Scatchard plot revealed two classes of receptors with dissociation constants of 3.2 X 10(-6) M and 4.7 X 10(-5) M.

L5 ANSWER 1 OF 13 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2006:362835 CAPLUS
DOCUMENT NUMBER: 144:371890
TITLE: Manufacture of glycosaminoglycan fractions, removal of impurities form glycosaminoglycan fractions, and agents for them
INVENTOR(S): Koyama, Hiroshi; Imamura, Kimihiro; Koshinbo, Akira; Onuki, Yoji
PATENT ASSIGNEE(S): Seikagaku Kogyo Co., Ltd., Japan
SOURCE: Jpn. Kokai Tokkyo Koho, 12 pp.
CODEN: JKXXAF
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| JP 2006104461 | A2 | 20060420 | JP 2005-261689 | 20050909 |
| PRIORITY APPLN. INFO.: | | | JP 2004-264466 A | 20040910 |

AB The manufacturing method of glycosaminoglycan fractions with nucleic acid content (ethidium bromide method) <0.08 μg/mg, Fe content (atomic absorption measurement) ≤0.07 ppm, and protein content (Rowry method) ≤0.4% includes contacting the fraction solns. with activated charcoal, removing the activated charcoal, and isolating. Thus, preparing a fraction containing hyaluronic acid (I) from cock's combs, dissolving in purified water, adding activated charcoal treated in an autoclave, stirring for 1 h, and filtering gave a fraction showing nucleic acid content <0.08 μg/mg-I, Fe content 0.025-0.056 ppm, and protein content 0.004%.

L5 ANSWER 2 OF 13 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2005:1351123 CAPLUS
DOCUMENT NUMBER: 144:67172
TITLE: Method for purifying hyaluronic acid using calcium salt and phosphate salt, or calcium phosphate salt
INVENTOR(S): Kim, Tai-Hyo; Kim, Hui-Lae; Park, Sun-Young; Jang, Dong-Kwang
PATENT ASSIGNEE(S): T & Life System Co., Ltd., S. Korea
SOURCE: PCT Int. Appl., 24 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| WO 2005123934 | A1 | 20051229 | WO 2005-KR1821 | 20050615 |
| W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW | | | | |
| RW: BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG | | | | |
| PRIORITY APPLN. INFO.: | | | KR 2004-44450 A | 20040616 |

AB The present invention relates to a method for purifying hyaluronic acid using calcium salt and phosphate salt, or calcium phosphate salt, specifically to a method for purifying hyaluronic acid characterized by effectively removing lipids, nucleic acids and proteins from an extract or a culture solution which contains hyaluronic acid by treating with calcium salt and phosphate salt, or calcium phosphate salt. According to the method of the present invention, it is possible to purify hyaluronic acid more effectively, by avoiding a use of toxic organic materials as well as reducing addnl. processes.

REFERENCE COUNT: 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 3 OF 13 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2004:843644 CAPLUS
DOCUMENT NUMBER: 142:8216
TITLE: Purification of high molecular weight hyaluronic acid
INVENTOR(S): Kim, Duk Hee; Yang, Chang Mo; Choe, Sun Ah; Kim, Moo Sung; Kim, Seung Jung
PATENT ASSIGNEE(S): Pacific Co., Ltd, S. Korea
SOURCE: Repub. Korea, No pp. given
CODEN: KRXXFC
DOCUMENT TYPE: Patent
LANGUAGE: Korean
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| KR 149793 | B1 | 19980817 | KR 1995-66342 | 19951229 |

PRIORITY APPLN. INFO.: KR 1995-66342 19951229

AB High mol. weight of hyaluronic acid is purified by effectively removing nucleic acid, protein , exothermic material and low mol. weight of hyaluronic acid. Thus, 1 g crude hyaluronic acid is dissolved in 1 g H2O, followed by addition of 50 g polypropylene or hydrophobic polymer such as polyethylene, modified polypropylene or polystyrene at room temperature for 2 h and filtered, then 50 g active alumina is added in the filtrate, and stirred at 10-15° for 3 h, separated, adjusted to pH 7.0, filtered again. 40 g sodium chloride is added in the obtained filtrate, and precipitated with 2 g ethanol. The ppts. is separated and washed by 95 % ethanol, and vacuum-dried to give 0.7 g of hyaluronic acid sodium salt.

L5 ANSWER 4 OF 13 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2003:376293 CAPLUS
DOCUMENT NUMBER: 138:380406
TITLE: Recombinant expression of bacterial hyaluronan synthase genes in Bacillus and hyaluronic acid production
INVENTOR(S): Deangelis, Paul L.; Weigel, Paul H.; Kumari, Kshama
PATENT ASSIGNEE(S): University of Oklahoma Board of Regents, USA
SOURCE: U.S. Pat. Appl. Publ., 79 pp., Cont.-in-part of U.S. Ser. No. 469,200.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 23
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|

| | | | | | |
|---|---|---|---|---|---|
| US 2003092118 | A1 | 20030515 | US 2002-172527 | | 20020613 |
| US 6951743 | B2 | 20051004 | | | |
| EP 1522579 | A2 | 20050413 | EP 2004-29227 | | 19981030 |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI, CY | | | | | |
| ES 2235378 | T3 | 20050701 | ES 1998-957450 | | 19981030 |
| US 6833264 | B1 | 20041221 | US 1999-469200 | | 19991221 |
| US 2002160489 | A1 | 20021031 | US 2001-879959 | | 20010912 |
| CA 2451443 | AA | 20030724 | CA 2002-2451443 | | 20020613 |
| WO 2003060063 | A2 | 20030724 | WO 2002-US18915 | | 20020613 |
| WO 2003060063 | A3 | 20040916 | | | |
| W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW | | | | | |
| RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG | | | | | |
| AU 2002365206 | A1 | 20030730 | AU 2002-365206 | | 20020613 |
| EP 1481052 | A2 | 20041201 | EP 2002-804804 | | 20020613 |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI, CY, TR | | | | | |
| JP 2005514059 | T2 | 20050519 | JP 2003-560150 | | 20020613 |
| CN 1620511 | A | 20050525 | CN 2002-813370 | | 20020613 |
| US 2003113845 | A1 | 20030619 | US 2002-217613 | | 20020812 |
| US 6987023 | B2 | 20060117 | | | |
| US 2005287646 | A1 | 20051229 | US 2005-120422 | | 20050502 |
| US 7029880 | B2 | 20060418 | | | |
| US 2005266460 | A1 | 20051201 | US 2005-124215 | | 20050509 |
| PRIORITY APPLN. INFO.: | | | US 1997-64435P | P | 19971031 |
| | | | US 1998-178851 | B1 | 19981026 |
| | | | US 1999-469200 | A2 | 19991221 |
| | | | US 2001-297744P | P | 20010613 |
| | | | US 2001-297788P | P | 20010613 |
| | | | US 1997-899040 | B2 | 19970723 |
| | | | US 1998-80414P | P | 19980402 |
| | | | EP 1998-957450 | A3 | 19981030 |
| | | | US 1999-283402 | B1 | 19990401 |
| | | | US 2001-879959 | A1 | 20010912 |
| | | | WO 2002-US18915 | W | 20020613 |
| | | | US 2002-217613 | A1 | 20020812 |

AB The present invention relates to a recombinant Bacillus host cell containing a recombinant vector including a nucleic acid segment having a coding region segment encoding enzymically active hyaluronan synthase (HAS). The recombinant Bacillus host cell is utilized in methods for producing secreted hyaluronic acid (HA) that is further extracted and purified. The invention claims use of nucleic acid and protein sequences for HAS from Streptococcus uberis, Streptococcus pyogenes, and Pasteurella multocida. Methods for HA production include high-level expression of hyaluronan synthase from Bacillus-compatible promoters, use of mRNA stabilizing or destabilizing elements, and enhanced production of UDP-glucuronic acid and/or UDP-N-acetylglucosamine in the recombinant host cell through use of active UDP-sugar precursor biosynthetic enzyme genes. HA with mol. weight of .apprx.107 Daltons can be produced by recombinant expression of hyaluronan synthase.

L5 ANSWER 5 OF 13 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2002:234967 CAPLUS
DOCUMENT NUMBER: 138:56158
TITLE: Novel ion exchange resin for purification of

hyaluronic acid
AUTHOR(S): Li, Run; Ni, Hangsheng; Luo, Min; He, Yanli
CORPORATE SOURCE: Department of Chemistry, Tsinghua University, Beijing, 100084, Peop. Rep. China
SOURCE: Zhongguo Yiyao Gongye Zazhi (2002), 33(1), 16-18
CODEN: ZYGZEA; ISSN: 1001-8255
PUBLISHER: Zhongguo Yiyao Gongye Zazhi Bianjibu
DOCUMENT TYPE: Journal
LANGUAGE: Chinese
AB A novel ion exchanger prepared from a strong anion resin modified with histidine was used for purifying crude hyaluronic acid to achieve a successful separation from protein and nucleic acids.

L5 ANSWER 6 OF 13 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1993:250955 CAPLUS
DOCUMENT NUMBER: 118:250955
TITLE: Simultaneous preparation and quantitation of proteoglycans by precipitation with Alcian blue
AUTHOR(S): Bjoernsson, Sven
CORPORATE SOURCE: Dep. Clin. Chem., Univ. Hosp., Lund, S-221 85, Swed.
SOURCE: Analytical Biochemistry (1993), 210(2), 282-91
CODEN: ANBCA2; ISSN: 0003-2697
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Conditions for specific interaction between Alcian blue and proteoglycans were optimized by comparing the differential spectra of Alcian blue obtained with purified chondroitin sulfate dissolved in water with the spectra obtained with nasal cartilage proteoglycans dissolved in synovial fluid. A method was then designed that provides specific precipitation of proteoglycans or glycosaminoglycans in 4M guanidine-HCl in the presence of protein, hyaluronic acid, or nucleic acids. The specificity is achieved by using a low pH in combination with detergent and high salt concentration Stepwise addition of reagents is necessary to avoid binding to Alcian blue to proteins and nucleic acids. All polyanions, except polysulfates, are first neutralized by lowering the pH to 1.5. By including detergent in this step, the hydrophobic protein regions are blocked and not accessible for binding with the dye. These regions could otherwise bind Alcian blue by hydrophobic interaction. When the Alcian blue reagent is added after, only the polysulfated mols. will remain charged and free to interact with Alcian blue. At least 0.4M guanidine-HCl is required to abolish the neg. interference by proteins. All sulfated glycosaminoglycans are precipitated at 0.4M guanidine-HCl. With increasing guanidine-HCl concns., the different glycosaminoglycans are precipitated in accordance with the critical electrolyte concentration of the resp. glycosaminoglycan. The Alcian blue precipitation can be performed at different concns. of guanidine-HCl in order to sep. different classes of proteoglycans. Excess dye and contaminating proteins are removed by a wash in a DMSO-MgCl2 solution and the precipitate is dissolved in a mixture of guanidine-HCl and propanol. For quantitation, the absorbance is recorded in a microplate reader with the 600-nm filter, the assay being linear between 0.5 and 20 μg proteoglycan. Since no digestion of samples with protease is needed, the proteoglycans are recovered in native form. The proteoglycan-Alcian blue complexes dissociate in the guanidine-HCl/propanol mixture and the proteoglycans can be selectively precipitated with propanol. The dye is used for quantitation and the proteoglycans can be utilized for further anal.

L5 ANSWER 7 OF 13 CAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 1992:529972 CAPLUS
DOCUMENT NUMBER: 117:129972
TITLE: Manufacture of hyaluronic acid by continuous fermentation of Streptococcus
INVENTOR(S): Ellwood, Derek Clifford; Evans, Charles Gervase Thorngate; Dunn, Geoffrey Michael; McInnes, Neil; Yeo, Richard Grenville; Smith, Keith James
PATENT ASSIGNEE(S): Fermentech Ltd., UK
SOURCE: PCT Int. Appl., 27 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| WO 9208799 | A1 | 19920529 | WO 1991-GB1927 | 19911104 |
| W: JP, US | | | | |
| RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, NL, SE | | | | |
| EP 556213 | A1 | 19930825 | EP 1991-918590 | 19911104 |
| EP 556213 | B1 | 19980318 | | |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE | | | | |
| JP 06505146 | T2 | 19940616 | JP 1991-517749 | 19911104 |
| JP 3159983 | B2 | 20010423 | | |
| AT 164185 | E | 19980415 | AT 1991-918590 | 19911104 |
| ES 2115620 | T3 | 19980701 | ES 1991-918590 | 19911104 |
| US 5411874 | A | 19950502 | US 1993-50046 | 19930706 |
| US 5563051 | A | 19961008 | US 1995-377265 | 19950124 |

PRIORITY APPLN. INFO.:

| | | |
|---|---|---|
| GB 1990-24223 | A | 19901107 |
| WO 1991-GB1927 | W | 19911104 |
| US 1993-50046 | A1 | 19930706 |

AB A high mol.-weight hyaluronic acid free of toxic products and suitable for pharmaceuticals is manufactured by continuous culture of Streptococcus equi. S. equi was grown in a yeast extract/salts medium at 37° with medium replaced at 0.07 vol/h with aeration maintaining O tension at 0.2% saturation Eluted medium contained 2.5 g hyaluronic acid/L; this was killed and detergent extracted with formalin at 1% volume/volume and 0.025% sodium dodecyl sulfate 0.025% w/v with a contact time of 16 h. Biomass residue was removed by filtration and the filtrate was purified by diafiltration and nucleic acids in the retentate precipitated with cetyl pyridinium bromide. After filtration to remove the precipitate the hyaluronic acid was precipitated with isopropanol. Average mol. weight of the hyaluronic acid was 1.6-2.5+106 and it had <0.2% protein and <0.15% nucleotides. The viscosity of a 1% solution was 159 Pa.s at zero shear and <1 Pa.s at 1000 s-1.

L5 ANSWER 8 OF 13 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1990:181782 CAPLUS
DOCUMENT NUMBER: 112:181782
TITLE: Alumina for purification of hyaluronic acid
INVENTOR(S): Kono, Susumu; Nishimura, Hisao; Ishii, Hisao; Chiba, Susumu; Kitagawa, Hiroyuki; Miyoshi, Teruzo
PATENT ASSIGNEE(S): Denki Kagaku Kogyo K. K., Japan
SOURCE: Jpn. Kokai Tokkyo Koho, 5 pp.
CODEN: JKXXAF
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|

JP 01313503 A2 19891219 JP 1988-144728 19880614
PRIORITY APPLN. INFO.: JP 1988-144728 19880614
AB Hyaluronic acid (I) solution is purified by using alumina as absorbent for preparing pure product useful as moisturizing agents for cosmetics and pharmaceuticals. Thus, 500 mL hollow-fiber predialized 610 g/L I solution was combined with 15 g NaCl, adjusted to pH 7, and combined with 2 L acetone to give a precipitate which was washed with EtOH and dried. Mixing an aqueous solution of the I (protein content 0.65%, pyrogen content 3 mg/mg) with chromatog. alumina, decanting, filtering with ultrafiltration membrane, desalting using EtOH, and drying gave pure I with protein content 0.01%, nucleic acid content 0%, pyrogen content 50 pg/mg I-salt and no pyrogenic reaction.

L5 ANSWER 9 OF 13 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1985:558422 CAPLUS
DOCUMENT NUMBER: 103:158422
TITLE: Basic protein-glycosaminoglycan interaction as a possible primary cause of vascular and cardiac damage
AUTHOR(S): Tedeschi, Guido G.; Cingolani, Rosalia Tacconi
CORPORATE SOURCE: Sch. Clin. Chem. Bacteriol., Univ. Camerino, Camerino, 62032, Italy
SOURCE: IRCS Medical Science (1985), 13(6), 514
CODEN: IMSCE2; ISSN: 0268-8220
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The addition of hyaluronic acid and/or chondroitin sulfate to histone or human globin solns. caused the immediate formation of voluminous aggregates of eosinophil particles and fibrouis material with strong affinity towards Alcian blue, insol. in the pH range 2-13. The addition of hyaluronic acid and/or chondroitin sulfate solns. to β-hemolytic Streptococcus group A cultures from the beginning of the incubation or at various time intervals, caused rapid agglutination and sedimentation, lysis, and the appearance of granular material which condensed in large aggregates held together by fibrous structures embedding the bacterial bodies. A similar addition to the 4500 + g supernatant gave rise to a precipitate containing a small number of altered bacteria and a relatively large amount of low mol. weight nucleic acids. No nucleic acids in a detectable amount were present in the precipitate produced following addition of hyaluronic acid and/or chondroitin sulfate to the 0.45 μm filtrate of the cultures. The expts. where saline solns. of purified histone proteins and glycosaminoglycans were used indicate that the precipitation may be attributed to the presence of basic proteins within the cultures. The initial formation of particulate material in vivo accompanied by deposit at the cardiac and vascular level, could be attributed to interaction between basic proteins and glycosaminoglycans, independent of the presence of lipid components. This process could be enhanced during the course of inflammation, especially if it was caused or accompanied by bacterial growth.

L5 ANSWER 10 OF 13 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1985:486524 CAPLUS
DOCUMENT NUMBER: 103:86524
TITLE: Ultrapure hyaluronic acid
INVENTOR(S): Brown, Karen K.; Ruiz, Linda L. Clem; Van de Rijn, Ivo
PATENT ASSIGNEE(S): Miles Laboratories, Inc. , USA
SOURCE: Eur. Pat. Appl., 30 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO. KIND DATE APPLICATION NO. DATE

| | | | | | |
|---|---|---|---|---|---|
| EP 144019 | A2 | 19850612 | EP 1984-113716 | | 19841114 |
| EP 144019 | A3 | 19861008 | | | |
| EP 144019 | B1 | 19900613 | | | |
| R: AT, BE, CH, DE, FR, GB, IT, LI, NL, SE | | | | | |
| NO 8404493 | A | 19850528 | NO 1984-4493 | | 19841109 |
| NO 161573 | B | 19890522 | | | |
| NO 161573 | C | 19890830 | | | |
| AT 53607 | E | 19900615 | AT 1984-113716 | | 19841114 |
| ZA 8409025 | A | 19850731 | ZA 1984-9025 | | 19841120 |
| FI 8404597 | A | 19850526 | FI 1984-4597 | | 19841122 |
| FI 79555 | B | 19890929 | | | |
| FI 79555 | C | 19900110 | | | |
| AU 8435806 | A1 | 19850530 | AU 1984-35806 | | 19841122 |
| AU 573768 | B2 | 19880623 | | | |
| CA 1270219 | A1 | 19900612 | CA 1984-468446 | | 19841122 |
| DK 8405588 | A | 19850526 | DK 1984-5588 | | 19841123 |
| HU 36180 | O | 19850828 | HU 1984-4359 | | 19841123 |
| HU 192617 | B | 19870629 | | | |
| ES 537938 | A1 | 19851216 | ES 1984-537938 | | 19841123 |
| IL 73605 | A1 | 19920329 | IL 1984-73605 | | 19841123 |
| JP 60133894 | A2 | 19850717 | JP 1984-247046 | | 19841124 |
| US 4782046 | A | 19881101 | US 1986-910246 | | 19860918 |
| PRIORITY APPLN. INFO.: | | | US 1983-555224 | A | 19831125 |
| | | | EP 1984-113716 | A | 19841114 |
| | | | US 1985-801973 | A1 | 19851126 |

AB Hyaluronic acid [9004-61-9] is produced by cultivation of Streptococcus equi and is purified by a process comprising repeated alc. precipitation and solubilization in water, followed by filtration through nitrocellulose. The preparation has a high average mol. weight, with >98% measuring between 2,000,000 and 4,000,000 and has <0.10 mg protein and <5 μg nucleic acid/mL. It is nonreactive and reversed lameness and stiffness when injected into diseased joints in horses.

L5 ANSWER 11 OF 13 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1985:452730 CAPLUS
DOCUMENT NUMBER: 103:52730
TITLE: Use of ultrapure hyaluronic acid to improve animal joint function
INVENTOR(S): Brown, Karen K.; Cooper, Harold
PATENT ASSIGNEE(S): Miles Laboratories, Inc. , USA
SOURCE: Eur. Pat. Appl., 29 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | | DATE |
|---|---|---|---|---|---|
| EP 143393 | A2 | 19850605 | EP 1984-113715 | | 19841114 |
| EP 143393 | A3 | 19861008 | | | |
| R: AT, BE, CH, DE, FR, GB, IT, LI, NL, SE | | | | | |
| NO 8404462 | A | 19850528 | NO 1984-4462 | | 19841108 |
| NO 160722 | B | 19890213 | | | |
| NO 160722 | C | 19890524 | | | |
| FI 8404598 | A | 19850526 | FI 1984-4598 | | 19841122 |
| AU 8435807 | A1 | 19850530 | AU 1984-35807 | | 19841122 |
| AU 574405 | B2 | 19880707 | | | |
| PRIORITY APPLN. INFO.: | | | US 1983-555310 | A | 19831125 |

AB Sterile, purified hyaluronic acid [9004-61-9] prepns. substantially free of proteins and

nucleic acids and having a closely controlled high-mol.-weight distribution are used as joint replacement fluids. The hyaluronic acid is bacterial derived. E.g., Streptococcus equi was fermented at pH 7.0-7.2 24-120 h at 37° in a medium containing no extraneous proteins and to which dextrose was added as the C source. The pH was allowed to drop to 6.5-6.8 .apprx.12 h before harvest. At harvest ≥0.01% Na lauryl sulfate was added followed by hexadecyltrimethylammonium bromide. The resultant precipitate was solubilized in 2M CaCl2 and the resulting suspension centrifuged to remove the precipitate Purified hyaluronic acid was recovered from the supernatant. The resultant hyaluronic acid was an acceptable joint replacement fluid in horses.

L5 ANSWER 12 OF 13 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1969:487745 CAPLUS
DOCUMENT NUMBER: 71:87745
TITLE: Protein-polysaccharides from pig laryngeal cartilage. Extraction and purification
AUTHOR(S): Tsiganos, Constantine P.; Muir, Helen
CORPORATE SOURCE: Kennedy Inst. Rheumatol., London, UK
SOURCE: Biochemical Journal (1969), 113(5), 879-84
CODEN: BIJOAK; ISSN: 0264-6021
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Protein-polysaccharides of chondroitin sulfate were extracted from fresh laryngeal cartilage at pH 6.8 by 2 procedures. Procedure 1 consisted of brief low-speed homogenization in 0.15M (iso-osmotic) NaOAc and procedure 2 consisted of longer homogenization followed by prolonged extraction in 10% CaCl2 solution The protein-polysaccharides in both exts. were isolated and purified by precipitation with 9-aminoacridine-HCl. They were free from serum proteins, collagen, and nucleic acids and also of degradative enzymes. The absence of such enzymes was shown by viscosity measurements on solns. of protein-polysaccharides incubated for up to 24 hrs. at pH 4 and 6.8. Mannose, glucose, or fucose was not detected by paper chromatog. and only traces of sialic acid were present. The yield with procedure 2 was twice that with procedure 1 and the products differed in their protein and glucosamine contents. Hyaluronic acid was unlikely to have been precipitated at an acid pH, so the glucosamine was attributed to keratan sulfate, as serum proteins were absent. There was no free keratan sulfate in the preparation Both prepns. were heterogeneous in the ultracentrifuge, showing at least 3 components.

L5 ANSWER 13 OF 13 MEDLINE on STN
ACCESSION NUMBER: 93289951 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8512063
TITLE: Simultaneous preparation and quantitation of proteoglycans by precipitation with alcian blue.
AUTHOR: Bjornsson S
CORPORATE SOURCE: Department of Clinical Chemistry, University Hospital, Lund, Sweden.
SOURCE: Analytical biochemistry, (1993 May 1) Vol. 210, No. 2, pp. 282-91.
Journal code: 0370535. ISSN: 0003-2697.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199307
ENTRY DATE: Entered STN: 23 Jul 1993
Last Updated on STN: 6 Feb 1998
Entered Medline: 9 Jul 1993
AB Conditions for specific interaction between Alcian blue and proteoglycans

were optimized by comparing the differential spectra of Alcian blue obtained with purified chondroitin sulfate dissolved in water with the spectra obtained with nasal cartilage proteoglycans dissolved in synovial fluid. A method was then designed that provides specific precipitation of proteoglycans or glycosaminoglycans in 4 M guanidine - HCl in the presence of protein, hyaluronic acid, or nucleic acids. The specificity is achieved by using a low pH in combination with detergent and high salt concentration. Stepwise addition of reagents is necessary to avoid binding of Alcian blue to proteins and nucleic acids. All polyanions, except polysulfates, are first neutralized by lowering the pH to 1.5. By including detergent in this step, the hydrophobic protein regions are blocked and not accessible for binding with the dye. These regions could otherwise bind Alcian blue by hydrophobic interaction. When the Alcian blue reagent is added after, only the polysulfated molecules will remain charged and free to interact with Alcian blue. At least 0.4 M guanidine-HCl is required to abolish the negative interference by proteins. All sulfated glycosaminoglycans are precipitated at 0.4 M guanidine-HCl. With increasing guanidine-HCl concentrations, the different glycosaminoglycans are precipitated in accordance with the critical electrolyte concentration of the respective glycosaminoglycan. The Alcian blue precipitation can be performed at different concentrations of guanidine-HCl in order to separate different classes of proteoglycans. Excess dye and contaminating proteins are removed by a wash in a DMSO-MgCl2 solution and the precipitate is dissolved in a mixture of guanidine-HCl and propanol. For quantitation, the absorbance is recorded in a microplate reader with the 600-nm filter, the assay being linear between 0.5 and 20 micrograms proteoglycan. Since no digestion of samples with protease is needed, the proteoglycans are recovered in native form. The proteoglycan-Alcian blue complexes dissociate in the guanidine-HCl/propanol mixture and the proteoglycans can be selectively precipitated with propanol. The dye is used for quantitation and the proteoglycans can be utilized for further analysis.

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     (FILE 'HOME' ENTERED AT 12:08:02 ON 06 JUL 2006)

     FILE 'CAPLUS, MEDLINE' ENTERED AT 12:08:15 ON 06 JUL 2006
L1             0 S GLYCOAMINOGLYCAN? (P) PROTEIN? (P) NUCLEIC ACID? (P) ULTRA?
L2             2 S GLYCOSAMINOGLYCAN? (P) PROTEIN? (P) NUCLEIC ACID? (P) ULTRA?
L3             2 S GLYCOSAMINOGLYCAN? (P) PROTEIN? (P) NUCLEIC ACID? (P) WAVELEN
L4            15 S GLYCOSAMINOGLYCAN? (P) PROTEIN? (P) NUCLEIC ACID? (P) PURIF?
L5            13 S HYALURONIC ACID? (P) PROTEIN? (P) NUCLEIC ACID? (P) PURIF?
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     (FILE 'HOME' ENTERED AT 12:08:02 ON 06 JUL 2006)

     FILE 'CAPLUS, MEDLINE' ENTERED AT 12:08:15 ON 06 JUL 2006
L1             0 S GLYCOAMINOGLYCAN? (P) PROTEIN? (P) NUCLEIC ACID? (P) ULTRA?
L2             2 S GLYCOSAMINOGLYCAN? (P) PROTEIN? (P) NUCLEIC ACID? (P) ULTRA?
L3             2 S GLYCOSAMINOGLYCAN? (P) PROTEIN? (P) NUCLEIC ACID? (P) WAVELEN
L4            15 S GLYCOSAMINOGLYCAN? (P) PROTEIN? (P) NUCLEIC ACID? (P) PURIF?
L5            13 S HYALURONIC ACID? (P) PROTEIN? (P) NUCLEIC ACID? (P) PURIF?
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L1 ANSWER 1 OF 12 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2004:885394 CAPLUS
DOCUMENT NUMBER: 142:133459
TITLE: Dried Rana catesbeiana extract having chondroitin sulfate
INVENTOR(S): Toida, Toshihiko; Han, Beom Su; Kim, Young Sik; Woo, Song Ji
PATENT ASSIGNEE(S): S. Korea
SOURCE: Repub. Korean Kongkae Taeho Kongbo, No pp. given
CODEN: KRXXA7
DOCUMENT TYPE: Patent
LANGUAGE: Korean
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| KR 2001017847 | A | 20010305 | KR 1999-33581 | 19990816 |

PRIORITY APPLN. INFO.: KR 1999-33581 19990816

AB Dried Rana catesbeiana extract and glycosaminoglycan segregated and purified from the dried extract are provided to effectively use the dried Rana catesbeiana extract for eye drops, rheumatism, new blood vessel growth control, osteoporosis, liver function improvement and climacterium by using the dried extract as an enzyme, protease. Dried Rana catesbeiana extract contains glycosaminoglycan having 6.0 of chondroitin, 26.9 of chondroitin sulfate C and 67.1 of chondroitin sulfate A. The manufacturing method of the dried Rana catesbeiana extract comprises the steps of: (1) dissolving the dried extract in buffer solution of pH 6.5-9.0 or NaOH solution of 0.05N; (2) adding protease like subtilisin in the mixture of the step (1); (3) treating the mixture of the step (2) with calcium chloride and 3-chloro-acetate to precipitate protein or nucleic acid; and (4) precipitating again the precipitate of the step (3) by ethanol and drying. The dried extract is dissolved in sodium chloride and precipitate complex is produced by adding quadrihydric ammonium. The precipitate complex is dissolved in a strong base and precipitated again by ethanol. Glycosaminoglycan is segregated for purifying from the dried Rana catesbeiana extract

L1 ANSWER 2 OF 12 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1993:250955 CAPLUS
DOCUMENT NUMBER: 118:250955
TITLE: Simultaneous preparation and quantitation of proteoglycans by precipitation with Alcian blue
AUTHOR(S): Bjoernsson, Sven
CORPORATE SOURCE: Dep. Clin. Chem., Univ. Hosp., Lund, S-221 85, Swed.
SOURCE: Analytical Biochemistry (1993), 210(2), 282-91
CODEN: ANBCA2; ISSN: 0003-2697
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Conditions for specific interaction between Alcian blue and proteoglycans were optimized by comparing the differential spectra of Alcian blue obtained with purified chondroitin sulfate dissolved in water with the spectra obtained with nasal cartilage proteoglycans dissolved in synovial fluid. A method was then designed that provides specific precipitation of proteoglycans or glycosaminoglycans in 4M guanidine-HCl in the presence of protein, hyaluronic acid, or nucleic acids. The specificity is achieved by using a low pH in combination with detergent and high salt concentration Stepwise addition of reagents is necessary to avoid binding to Alcian blue to proteins

and nucleic acids. All polyanions, except polysulfates, are first neutralized by lowering the pH to 1.5. By including detergent in this step, the hydrophobic protein regions are blocked and not accessible for binding with the dye. These regions could otherwise bind Alcian blue by hydrophobic interaction. When the Alcian blue reagent is added after, only the polysulfated mols. will remain charged and free to interact with Alcian blue. At least 0.4M guanidine-HCl is required to abolish the neg. interference by proteins. All sulfated glycosaminoglycans are precipitated at 0.4M guanidine-HCl. With increasing guanidine-HCl concns., the different glycosaminoglycans are precipitated in accordance with the critical electrolyte concentration of the resp. glycosaminoglycan. The Alcian blue precipitation can be performed at different concns. of guanidine-HCl in order to sep. different classes of proteoglycans. Excess dye and contaminating proteins are removed by a wash in a DMSO-MgCl2 solution and the precipitate is dissolved in a mixture of guanidine-HCl and propanol. For quantitation, the absorbance is recorded in a microplate reader with the 600-nm filter, the assay being linear between 0.5 and 20 μg proteoglycan. Since no digestion of samples with protease is needed, the proteoglycans are recovered in native form. The proteoglycan-Alcian blue complexes dissociate in the guanidine-HCl/propanol mixture and the proteoglycans can be selectively precipitated with propanol. The dye is used for quantitation and the proteoglycans can be utilized for further anal.

L1 ANSWER 3 OF 12 CAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 1992:125378 CAPLUS
DOCUMENT NUMBER: 116:125378
TITLE: High molecular weight mucin-like glycoproteins of the bovine interphotoreceptor matrix
AUTHOR(S): Plantner, James J.
CORPORATE SOURCE: Sch. Med., Case West. Reserve Univ., Cleveland, OH, 44106, USA
SOURCE: Experimental Eye Research (1992), 54(1), 113-25
CODEN: EXERA6; ISSN: 0014-4835
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A very high-mol. weight mucin-like glycoprotein was isolated by gel filtration of interphotoreceptor matrix (IPM) from fresh bovine eyes and purified to apparent homogeneity by CsCl/guanidine hydrochloride (GuHCl) equilibrium d. gradient centrifugation. Although a mol. weight in excess of 107 Da is suggested by gel filtration, the presence of SDS or GuHCl did not alter its elution position, indicating that the large size was not simply due to aggregation. Treatment of this material with disulfide reagents, however, led to a decrease in mol. size. On a relative basis, substantially more of this glycoprotein is present in IPM prepared from retina than from retinal pigment epithelium. While the carbohydrate and amino acid composition are not those of a true mucin, the large size and many other properties are quite mucin-like. The carbohydrate composition suggests the presence of both N- and O-glycosidically linked sugar chains. The presence of a mucin-type O-glycosidic linkage is indicated by its suceptibility to alkaline cleavage, with concomitant loss of serine and threonine and increase in 240 nm absorbance; production of a fluorescent product on reaction with cyanoacetamide; lectin-binding properties; and production of N-acetylgalactosaminitol on alkaline borohydride elimination. This glycoprotein was digested by pronase and trypsin, confirming its protein nature, but was resistant to digestion with chondroitin ABC lyase, hyaluronidase and heparinase, as well as RNAase, indicating that these components were not present to any appreciable extent. ELISA for cartilage keratan sulfate was also neg. Centrifugation in CsCl/GuHCl gradients indicated a d. much lower than that

of a proteoglycan or nucleic acid as well. In vitro biosynthetic studies suggest that both retina and retinal pigment epithelium may be major sources of material in the IPM. The elution patterns of radioactivity were strikingly similar to the UV elution patterns of IPM. The medium from retinal incubations contained very high mol. weight material which was resistant to enzymes which hydrolyze glycosaminoglycans, suggesting that retina may be the source of this high-mol. weight, mucin-like glycoprotein.

L1 ANSWER 4 OF 12 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1988:91226 CAPLUS
DOCUMENT NUMBER: 108:91226
TITLE: Densitometric assay of nanogram quantities of proteoglycans precipitated on nitrocellulose membrane with safranin O
AUTHOR(S): Lammi, Mikko; Tammi, Markku
CORPORATE SOURCE: Dep. Anat., Univ. Kuopio, Kuopio, SF-70211, Finland
SOURCE: Analytical Biochemistry (1988), 168(2), 352-7
CODEN: ANBCA2; ISSN: 0003-2697
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Proteoglycan (PG) and glycosaminoglycan (GAG) samples corresponding to a min. of 10 ng uronic acid were reliably quantified as ppts. with the cationic dye Safranin O, collected by vacuum-aided filtration onto a cellulose acetate/nitrate membrane in a standard 96-well dot assay apparatus The reflectances of the precipitation dots were measured by automatic densitometric scanning of the membrane sheets. Standard GAGs produced reflectance values which were related to the number of anionic groups per unit disaccharide; hyaluronate and keratan sulfate gave lower values while heparin yielded values higher than those of chondroitin sulfates. The presence of 8M urea, 1% Triton X 100, 30% sucrose, 0.02% NaN3, or mixts. of proteinase inhibitors and various buffers did not markedly influence the reflectances, while 4M guanidinium chloride and 3M CsCl reduced the sensitivity of the assay to 30-50 ng. Samples containing SDS were not applicable because SDS precipitated with Safranin O. Proteins showed virtually no response, while nucleic acids gave significant although smaller reflectances than GAGs. Owing to its marked sensitivity and convenience, the method is particularly suitable for the detection of PGs during their preparative purifn. and fractionation as well as in various anal. assays.

L1 ANSWER 5 OF 12 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1986:605107 CAPLUS
DOCUMENT NUMBER: 105:205107
TITLE: Renal plasma membrane receptors for certain modified serum albumins. Evidence for participation of a heparin receptor
AUTHOR(S): Ranganathan, Perungavur N.; Mego, John L.
CORPORATE SOURCE: Biol. Dep., Univ. Alabama, University, AL, 35486, USA
SOURCE: Biochemical Journal (1986), 239(3), 537-43
CODEN: BIJOAK; ISSN: 0306-3275
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The binding of formaldehyde-treated (f-alb), reduced-carboxymethylated (ac-alb) or reduced-acetamidated (am-alb) bovine serum albumins to purified rat renal plasma membranes was studied. Radioiodinated f-alb or ac-alb bound to kidney membranes, whereas am-alb neither bound significantly nor competed with f-alb binding to kidney membranes. The binding was specific, saturable, and heat- and proteinase -sensitive. Competition studies showed that f-alb and ac-alb sites may be the same on these membranes. To determine the role played by charge in binding, competition expts. with polyanions were performed. Polyanions, such as nucleic acid or glycosaminoglycans, were

effective competitors of f-alb binding to cell membranes. Heparin was especially inhibitory, being several-fold more so than chondroitin sulfate. Completely reduced and carboxymethylated albumin was a better competitor than its partially modified counterpart. Furthermore, f-alb was a significant competitor of [35S]heparin binding to kidney membranes. Also, partially purified heparin receptor demonstrated specific binding of 125I-f-alb. The data suggested that a heparin receptor is responsible for binding and internalization of i.v. injected f-alb. A Scatchard plot revealed 2 classes of receptors with dissociation consts. of 3.2 + 10-6 and 4.7 + 10-5 M.

L1 ANSWER 6 OF 12 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1985:558422 CAPLUS
DOCUMENT NUMBER: 103:158422
TITLE: Basic protein-glycosaminoglycan interaction as a possible primary cause of vascular and cardiac damage
AUTHOR(S): Tedeschi, Guido G.; Cingolani, Rosalia Tacconi
CORPORATE SOURCE: Sch. Clin. Chem. Bacteriol., Univ. Camerino, Camerino, 62032, Italy
SOURCE: IRCS Medical Science (1985), 13(6), 514
CODEN: IMSCE2; ISSN: 0268-8220
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The addition of hyaluronic acid and/or chondroitin sulfate to histone or human globin solns. caused the immediate formation of voluminous aggregates of eosinophil particles and fibrouis material with strong affinity towards Alcian blue, insol. in the pH range 2-13. The addition of hyaluronic acid and/or chondroitin sulfate solns. to β-hemolytic Streptococcus group A cultures from the beginning of the incubation or at various time intervals, caused rapid agglutination and sedimentation, lysis, and the appearance of granular material which condensed in large aggregates held together by fibrous structures embedding the bacterial bodies. A similar addition to the 4500 + g supernatant gave rise to a precipitate containing a small number of altered bacteria and a relatively large amount of low mol. weight nucleic acids. No nucleic acids in a detectable amount were present in the precipitate produced following addition of hyaluronic acid and/or chondroitin sulfate to the 0.45 μm filtrate of the cultures. The expts. where saline solns. of purified histone proteins and glycosaminoglycans were used indicate that the precipitation may be attributed to the presence of basic proteins within the cultures. The initial formation of particulate material in vivo accompanied by deposit at the cardiac and vascular level, could be attributed to interaction between basic proteins and glycosaminoglycans, independent of the presence of lipid components. This process could be enhanced during the course of inflammation, especially if it was caused or accompanied by bacterial growth.

L1 ANSWER 7 OF 12 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1969:487745 CAPLUS
DOCUMENT NUMBER: 71:87745
TITLE: Protein-polysaccharides from pig laryngeal cartilage. Extraction and purification
AUTHOR(S): Tsiganos, Constantine P.; Muir, Helen
CORPORATE SOURCE: Kennedy Inst. Rheumatol., London, UK
SOURCE: Biochemical Journal (1969), 113(5), 879-84
CODEN: BIJOAK; ISSN: 0264-6021
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Protein-polysaccharides of chondroitin sulfate were extracted from fresh laryngeal cartilage at pH 6.8 by 2 procedures. Procedure 1 consisted of brief low-speed homogenization in 0.15M (iso-osmotic) NaOAc and procedure 2 consisted of longer homogenization followed by prolonged

extraction in 10% CaCl2 solution The protein-polysaccharides in both exts. were isolated and purified by precipitation with 9-aminoacridine-HCl. They were free from serum proteins, collagen, and nucleic acids and also of degradative enzymes. The absence of such enzymes was shown by viscosity measurements on solns. of protein-polysaccharides incubated for up to 24 hrs. at pH 4 and 6.8. Mannose, glucose, or fucose was not detected by paper chromatog. and only traces of sialic acid were present. The yield with procedure 2 was twice that with procedure 1 and the products differed in their protein and glucosamine contents. Hyaluronic acid was unlikely to have been precipitated at an acid pH, so the glucosamine was attributed to keratan sulfate, as serum proteins were absent. There was no free keratan sulfate in the preparation Both prepns. were heterogeneous in the ultracentrifuge, showing at least 3 components.

L1 ANSWER 8 OF 12 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1969:8827 CAPLUS
DOCUMENT NUMBER: 70:8827
TITLE: Purification and characterization of sulfated glycoproteins and hyaluronidase-resistant mucopolysaccharides from dog gastric mucosa
AUTHOR(S): Pamer, Treva; Glass, George B.; Horowitz, Martin I.
CORPORATE SOURCE: New York Med. Coll., New York, NY, USA
SOURCE: Biochemistry (1968), 7(11), 3821-9
CODEN: BICHAW; ISSN: 0006-2960
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Sulfated glycoproteins were purified from papain-digested mucosal scrapings of the dog fundic stomach. The acidic fraction, isolated by EtOH and cetylpyridinium chloride pptns., was freed of nucleic acids by precipitation at pH 1.5 and of digestible mucopolysaccharides by treatment with testicular hyaluronidase. After gel filtration on Bio-Gel P-30 and Sephadex G-100, final separation of three sulfated glycoprotein fractions was achieved by elution from DEAE-Sephadex with NaCl. Each fraction exhibited a single band on cellulose acetate electrophoresis at pH 3 and 9. The 3 fractions were similar in having equimolar ratios of hexosamine and galactose, the major carbohydrate components, and in having glucosamine and galactosamine in equimolar ratios. SO42- was present in each fraction. Fucose and sialic acid content decreased with increasing molarity of eluting salt. Total protein ranged from 10.9 to 12%. Threonine, serine, proline, and valine constituted 88% of the total amino acid residues while aromatic and S amino acids were present only in trace quantities. Two hyaluronidase-resistant mucopolysaccharides were partially purified by Cu precipitation and tentatively identified as chondroitin sulfate-B and heparitin sulfate on the basis of their reactions in the carbazole and orcinol assays for uronic acids.

L1 ANSWER 9 OF 12 MEDLINE on STN
ACCESSION NUMBER: 93289951 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8512063
TITLE: Simultaneous preparation and quantitation of proteoglycans by precipitation with alcian blue.
AUTHOR: Bjornsson S
CORPORATE SOURCE: Department of Clinical Chemistry, University Hospital, Lund, Sweden.
SOURCE: Analytical biochemistry, (1993 May 1) Vol. 210, No. 2, pp. 282-91.
Journal code: 0370535. ISSN: 0003-2697.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199307

ENTRY DATE: Entered STN: 23 Jul 1993
Last Updated on STN: 6 Feb 1998
Entered Medline: 9 Jul 1993

AB Conditions for specific interaction between Alcian blue and proteoglycans were optimized by comparing the differential spectra of Alcian blue obtained with purified chondroitin sulfate dissolved in water with the spectra obtained with nasal cartilage proteoglycans dissolved in synovial fluid. A method was then designed that provides specific precipitation of proteoglycans or glycosaminoglycans in 4 M guanidine - HCl in the presence of protein, hyaluronic acid, or nucleic acids. The specificity is achieved by using a low pH in combination with detergent and high salt concentration. Stepwise addition of reagents is necessary to avoid binding of Alcian blue to proteins and nucleic acids. All polyanions, except polysulfates, are first neutralized by lowering the pH to 1.5. By including detergent in this step, the hydrophobic protein regions are blocked and not accessible for binding with the dye. These regions could otherwise bind Alcian blue by hydrophobic interaction. When the Alcian blue reagent is added after, only the polysulfated molecules will remain charged and free to interact with Alcian blue. At least 0.4 M guanidine-HCl is required to abolish the negative interference by proteins. All sulfated glycosaminoglycans are precipitated at 0.4 M guanidine-HCl. With increasing guanidine-HCl concentrations, the different glycosaminoglycans are precipitated in accordance with the critical electrolyte concentration of the respective glycosaminoglycan. The Alcian blue precipitation can be performed at different concentrations of guanidine-HCl in order to separate different classes of proteoglycans. Excess dye and contaminating proteins are removed by a wash in a DMSO-MgCl2 solution and the precipitate is dissolved in a mixture of guanidine-HCl and propanol. For quantitation, the absorbance is recorded in a microplate reader with the 600-nm filter, the assay being linear between 0.5 and 20 micrograms proteoglycan. Since no digestion of samples with protease is needed, the proteoglycans are recovered in native form. The proteoglycan-Alcian blue complexes dissociate in the guanidine-HCl/propanol mixture and the proteoglycans can be selectively precipitated with propanol. The dye is used for quantitation and the proteoglycans can be utilized for further analysis.

L1 ANSWER 10 OF 12 MEDLINE on STN
ACCESSION NUMBER: 92175078 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1541329
TITLE: High molecular weight mucin-like glycoproteins of the bovine interphotoreceptor matrix.
AUTHOR: Plantner J J
CORPORATE SOURCE: Lorand V. Johnson Laboratory for Research in Ophthalmology, Department of Surgery, Case Western Reserve University, Cleveland, OH 44106.
CONTRACT NUMBER: EY 06571 (NEI)
SOURCE: Experimental eye research, (1992 Jan) Vol. 54, No. 1, pp. 113-25.
Journal code: 0370707. ISSN: 0014-4835.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199204
ENTRY DATE: Entered STN: 24 Apr 1992
Last Updated on STN: 24 Apr 1992
Entered Medline: 8 Apr 1992

AB A very high molecular weight mucin-like glycoprotein was isolated by gel filtration of interphotoreceptor matrix (IPM) from fresh bovine eyes and purified to apparent homogeneity by cesium chloride/guanidine hydrochloride (GuHCl) equilibrium density gradient centrifugation.

Although a molecular weight in excess of 10(7) Da is suggested by gel filtration, the presence of SDS or GuHCl did not alter its elution position, indicating that the large size was not simply due to aggregation. Treatment of this material with disulfide reagents, however, led to a decrease in molecular size. On a relative basis, substantially more of this glycoprotein is present in IPM prepared from retina than from retinal pigment epithelium. While the carbohydrate and amino acid composition are not those of a true 'mucin', the large size and many other properties are quite 'mucin-like'. The carbohydrate composition suggests the presence of both N- and O-glycosidically linked sugar chains. The presence of a mucin-type O-glycosidic linkage is indicated by its susceptibility to alkaline cleavage, with concomitant loss of serine and threonine and increase in 240 nm absorbance; production of a fluorescent product upon reaction with cyanoacetamide; lectin binding properties; and production of N-acetylgalactosaminitol upon alkaline borohydride elimination. This glycoprotein was digested by pronase and trypsin, confirming its protein nature, but was resistant to digestion with chondroitin ABC lyase, hyaluronidase and heparinase, as well as RNAase, indicating that these components were not present to any appreciable extent. ELISA for cartilage keratan sulfate was also negative. Centrifugation in CsCl/GuHCl gradients indicated a density much lower than that of a proteoglycan or nucleic acid as well. In vitro biosynthetic studies suggest that both retina and retinal pigment epithelium may be major sources of material in the IPM. The elution patterns of radioactivity were strikingly similar to the UV elution patterns of IPM. The medium from retinal incubations contained very high molecular weight material which was resistant to enzymes which hydrolyse glycosaminoglycans, suggesting that retina may be the source of this high molecular weight, mucin-like glycoprotein.

L1 ANSWER 11 OF 12 MEDLINE on STN
ACCESSION NUMBER: 88207935 MEDLINE
DOCUMENT NUMBER: PubMed ID: 3129962
TITLE: Densitometric assay of nanogram quantities of proteoglycans precipitated on nitrocellulose membrane with Safranin O.
AUTHOR: Lammi M; Tammi M
CORPORATE SOURCE: Department of Anatomy, University of Kuopio, Finland.
SOURCE: Analytical biochemistry, (1988 Feb 1) Vol. 168, No. 2, pp. 352-7.
Journal code: 0370535. ISSN: 0003-2697.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198805
ENTRY DATE: Entered STN: 8 Mar 1990
Last Updated on STN: 8 Mar 1990
Entered Medline: 31 May 1988

AB Proteoglycan (PG) and glycosaminoglycan (GAG) samples corresponding to a minimum of 10 ng of uronic acid were reliably quantified as precipitates with the cationic dye Safranin O, collected by vacuum-aided filtration onto a cellulose acetate/nitrate membrane in a standard 96-well dot assay apparatus. The reflectances of the precipitation dots were measured by automatic densitometric scanning of the membrane sheets. Standard GAGs produced reflectance values which were related to the number of anionic groups per unit disaccharide; hyaluronate and keratan sulfate gave lower values while heparin yielded values higher than those of chondroitin sulfates. The presence of 8 M urea, 1% Triton X-100, 30% sucrose, 0.02% NaN3, or mixtures of proteinase inhibitors and various buffers did not markedly influence the reflectances, while 4 M guanidinium chloride and 3 M CsCl reduced the sensitivity of the assay to 30-50 ng. Samples containing sodium dodecyl sulfate (SDS) were not applicable because SDS precipitated with Safranin O. Proteins showed virtually no response, while nucleic acids gave

significant although smaller reflectances than GAGs. Owing to its marked sensitivity and convenience the method is particularly suitable for the detection of PGs during their preparative purification and fractionation as well as in various analytical assays.

L1 ANSWER 12 OF 12 MEDLINE on STN
ACCESSION NUMBER: 87156514 MEDLINE
DOCUMENT NUMBER: PubMed ID: 3030264
TITLE: Renal plasma membrane receptors for certain modified serum albumins. Evidence for participation of a heparin receptor.
AUTHOR: Ranganathan P N; Mego J L
SOURCE: The Biochemical journal, (1986 Nov 1) Vol. 239, No. 3, pp. 537-43.
Journal code: 2984726R. ISSN: 0264-6021.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198704
ENTRY DATE: Entered STN: 3 Mar 1990
Last Updated on STN: 3 Mar 1990
Entered Medline: 1 Apr 1987

AB Binding of formaldehyde-treated (f-alb), reduced-carboxymethylated (ac-alb) or reduced-acetamidated (am-alb) bovine serum albumins to purified rat renal plasma membranes was studied. Radioiodinated f-alb or ac-alb bound to kidney membranes while am-alb neither bound significantly nor competed with f-alb binding to kidney membranes. The binding was specific, saturable and heat- and proteinase -sensitive. Competition studies showed that f-alb and ac-alb sites may be the same on these membranes. To determine the role played by charge in binding, competition experiments with polyanions were performed. Polyanions such as nucleic acid or glycosaminoglycans were effective competitors of f-alb binding to cell membranes. Heparin was especially inhibitory, being several-fold more so than chondroitin sulphate. Completely reduced and carboxymethylated albumin was a better competitor than its partially modified counterpart. Furthermore, f-alb was a significant competitor of [35S]heparin binding to kidney membranes. Also, partially purified heparin receptor demonstrated specific binding of 125I-f-alb. These data suggest that a heparin receptor is responsible for binding and internalization of intravenously injected f-alb. A Scatchard plot revealed two classes of receptors with dissociation constants of 3.2 X 10(-6) M and 4.7 X 10(-5) M.

L2 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1992:125378 CAPLUS
DOCUMENT NUMBER: 116:125378
TITLE: High molecular weight mucin-like glycoproteins of the bovine interphotoreceptor matrix
AUTHOR(S): Plantner, James J.
CORPORATE SOURCE: Sch. Med., Case West. Reserve Univ., Cleveland, OH, 44106, USA
SOURCE: Experimental Eye Research (1992), 54(1), 113-25
CODEN: EXERA6; ISSN: 0014-4835
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A very high-mol. weight mucin-like glycoprotein was isolated by gel filtration of interphotoreceptor matrix (IPM) from fresh bovine eyes and purified to apparent homogeneity by CsCl/guanidine hydrochloride (GuHCl) equilibrium d. gradient centrifugation. Although a mol. weight in excess of 107 Da is suggested by gel filtration, the presence of SDS or GuHCl did not alter its elution position, indicating that the large size was not simply due to aggregation. Treatment of this material with disulfide reagents, however, led to a decrease in mol. size. On a relative basis, substantially more of this glycoprotein is present in IPM prepared from retina than from retinal pigment epithelium. While the carbohydrate and amino acid composition are not those of a true mucin, the large size and many other properties are quite mucin-like. The carbohydrate composition suggests the presence of both N- and O-glycosidically linked sugar chains. The presence of a mucin-type O-glycosidic linkage is indicated by its suceptibility to alkaline cleavage, with concomitant loss of serine and threonine and increase in 240 nm absorbance; production of a fluorescent product on reaction with cyanoacetamide; lectin-binding properties; and production of N-acetylgalactosaminitol on alkaline borohydride elimination. This glycoprotein was digested by pronase and trypsin, confirming its protein nature, but was resistant to digestion with chondroitin ABC lyase, hyaluronidase and heparinase, as well as RNAase, indicating that these components were not present to any appreciable extent. ELISA for cartilage keratan sulfate was also neg. Centrifugation in CsCl/GuHCl gradients indicated a d. much lower than that of a proteoglycan or nucleic acid as well. In vitro biosynthetic studies suggest that both retina and retinal pigment epithelium may be major sources of material in the IPM. The elution patterns of radioactivity were strikingly similar to the UV elution patterns of IPM. The medium from retinal incubations contained very high mol. weight material which was resistant to enzymes which hydrolyze glycosaminoglycans, suggesting that retina may be the source of this high-mol. weight, mucin-like glycoprotein.

L3 ANSWER 1 OF 12 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2004:885394 CAPLUS
DOCUMENT NUMBER: 142:133459
TITLE: Dried Rana catesbeiana extract having chondroitin sulfate
INVENTOR(S): Toida, Toshihiko; Han, Beom Su; Kim, Young Sik; Woo, Song Ji
PATENT ASSIGNEE(S): S. Korea
SOURCE: Repub. Korean Kongkae Taeho Kongbo, No pp. given
CODEN: KRXXA7
DOCUMENT TYPE: Patent
LANGUAGE: Korean
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| KR 2001017847 | A | 20010305 | KR 1999-33581 | 19990816 |

PRIORITY APPLN. INFO.: KR 1999-33581 19990816

AB Dried Rana catesbeiana extract and glycosaminoglycan segregated and purified from the dried extract are provided to effectively use the dried Rana catesbeiana extract for eye drops, rheumatism, new blood vessel growth control, osteoporosis, liver function improvement and climacterium by using the dried extract as an enzyme, protease. Dried Rana catesbeiana extract contains glycosaminoglycan having 6.0 of chondroitin, 26.9 of chondroitin sulfate C and 67.1 of chondroitin sulfate A. The manufacturing method of the dried Rana catesbeiana extract comprises the steps of: (1) dissolving the dried extract in buffer solution of pH 6.5-9.0 or NaOH solution of 0.05N; (2) adding protease like subtilisin in the mixture of the step (1); (3) treating the mixture of the step (2) with calcium chloride and 3-chloro-acetate to precipitate protein or nucleic acid; and (4) precipitating again the precipitate of the step (3) by ethanol and drying. The dried extract is dissolved in sodium chloride and precipitate complex is produced by adding quadrihydric ammonium. The precipitate complex is dissolved in a strong base and precipitated again by ethanol. Glycosaminoglycan is segregated for purifying from the dried Rana catesbeiana extract

L3 ANSWER 2 OF 12 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1993:250955 CAPLUS
DOCUMENT NUMBER: 118:250955
TITLE: Simultaneous preparation and quantitation of proteoglycans by precipitation with Alcian blue
AUTHOR(S): Bjoernsson, Sven
CORPORATE SOURCE: Dep. Clin. Chem., Univ. Hosp., Lund, S-221 85, Swed.
SOURCE: Analytical Biochemistry (1993), 210(2), 282-91
CODEN: ANBCA2; ISSN: 0003-2697
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Conditions for specific interaction between Alcian blue and proteoglycans were optimized by comparing the differential spectra of Alcian blue obtained with purified chondroitin sulfate dissolved in water with the spectra obtained with nasal cartilage proteoglycans dissolved in synovial fluid. A method was then designed that provides specific precipitation of proteoglycans or glycosaminoglycans in 4M guanidine-HCl in the presence of protein, hyaluronic acid, or nucleic acids. The specificity is achieved by using a low pH in combination with detergent and high salt concentration Stepwise addition of reagents is necessary to avoid binding to Alcian blue to proteins

and nucleic acids. All polyanions, except polysulfates, are first neutralized by lowering the pH to 1.5. By including detergent in this step, the hydrophobic protein regions are blocked and not accessible for binding with the dye. These regions could otherwise bind Alcian blue by hydrophobic interaction. When the Alcian blue reagent is added after, only the polysulfated mols. will remain charged and free to interact with Alcian blue. At least 0.4M guanidine-HCl is required to abolish the neg. interference by proteins. All sulfated glycosaminoglycans are precipitated at 0.4M guanidine-HCl. With increasing guanidine-HCl concns., the different glycosaminoglycans are precipitated in accordance with the critical electrolyte concentration of the resp. glycosaminoglycan. The Alcian blue precipitation can be performed at different concns. of guanidine-HCl in order to sep. different classes of proteoglycans. Excess dye and contaminating proteins are removed by a wash in a DMSO-MgCl2 solution and the precipitate is dissolved in a mixture of guanidine-HCl and propanol. For quantitation, the absorbance is recorded in a microplate reader with the 600-nm filter, the assay being linear between 0.5 and 20 μg proteoglycan. Since no digestion of samples with protease is needed, the proteoglycans are recovered in native form. The proteoglycan-Alcian blue complexes dissociate in the guanidine-HCl/propanol mixture and the proteoglycans can be selectively precipitated with propanol. The dye is used for quantitation and the proteoglycans can be utilized for further anal.

L3 ANSWER 3 OF 12 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1992:125378 CAPLUS
DOCUMENT NUMBER: 116:125378
TITLE: High molecular weight mucin-like glycoproteins of the bovine interphotoreceptor matrix
AUTHOR(S): Plantner, James J.
CORPORATE SOURCE: Sch. Med., Case West. Reserve Univ., Cleveland, OH, 44106, USA
SOURCE: Experimental Eye Research (1992), 54(1), 113-25
CODEN: EXERA6; ISSN: 0014-4835
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A very high-mol. weight mucin-like glycoprotein was isolated by gel filtration of interphotoreceptor matrix (IPM) from fresh bovine eyes and purified to apparent homogeneity by CsCl/guanidine hydrochloride (GuHCl) equilibrium d. gradient centrifugation. Although a mol. weight in excess of 107 Da is suggested by gel filtration, the presence of SDS or GuHCl did not alter its elution position, indicating that the large size was not simply due to aggregation. Treatment of this material with disulfide reagents, however, led to a decrease in mol. size. On a relative basis, substantially more of this glycoprotein is present in IPM prepared from retina than from retinal pigment epithelium. While the carbohydrate and amino acid composition are not those of a true mucin, the large size and many other properties are quite mucin-like. The carbohydrate composition suggests the presence of both N- and O-glycosidically linked sugar chains. The presence of a mucin-type O-glycosidic linkage is indicated by its suceptibility to alkaline cleavage, with concomitant loss of serine and threonine and increase in 240 nm absorbance; production of a fluorescent product on reaction with cyanoacetamide; lectin-binding properties; and production of N-acetylgalactosaminitol on alkaline borohydride elimination. This glycoprotein was digested by pronase and trypsin, confirming its protein nature, but was resistant to digestion with chondroitin ABC lyase, hyaluronidase and heparinase, as well as RNAase, indicating that these components were not present to any appreciable extent. ELISA for cartilage keratan sulfate was also neg. Centrifugation in CsCl/GuHCl gradients indicated a d. much lower than that

of a proteoglycan or nucleic acid as well. In vitro biosynthetic studies suggest that both retina and retinal pigment epithelium may be major sources of material in the IPM. The elution patterns of radioactivity were strikingly similar to the UV elution patterns of IPM. The medium from retinal incubations contained very high mol. weight material which was resistant to enzymes which hydrolyze glycosaminoglycans, suggesting that retina may be the source of this high-mol. weight, mucin-like glycoprotein.

L3 ANSWER 4 OF 12 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1988:91226 CAPLUS
DOCUMENT NUMBER: 108:91226
TITLE: Densitometric assay of nanogram quantities of proteoglycans precipitated on nitrocellulose membrane with safranin O
AUTHOR(S): Lammi, Mikko; Tammi, Markku
CORPORATE SOURCE: Dep. Anat., Univ. Kuopio, Kuopio, SF-70211, Finland
SOURCE: Analytical Biochemistry (1988), 168(2), 352-7
CODEN: ANBCA2; ISSN: 0003-2697
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Proteoglycan (PG) and glycosaminoglycan (GAG) samples corresponding to a min. of 10 ng uronic acid were reliably quantified as ppts. with the cationic dye Safranin O, collected by vacuum-aided filtration onto a cellulose acetate/nitrate membrane in a standard 96-well dot assay apparatus The reflectances of the precipitation dots were measured by automatic densitometric scanning of the membrane sheets. Standard GAGs produced reflectance values which were related to the number of anionic groups per unit disaccharide; hyaluronate and keratan sulfate gave lower values while heparin yielded values higher than those of chondroitin sulfates. The presence of 8M urea, 1% Triton X 100, 30% sucrose, 0.02% NaN3, or mixts. of proteinase inhibitors and various buffers did not markedly influence the reflectances, while 4M guanidinium chloride and 3M CsCl reduced the sensitivity of the assay to 30-50 ng. Samples containing SDS were not applicable because SDS precipitated with Safranin O. Proteins showed virtually no response, while nucleic acids gave significant although smaller reflectances than GAGs. Owing to its marked sensitivity and convenience, the method is particularly suitable for the detection of PGs during their preparative purifn. and fractionation as well as in various anal. assays.

L3 ANSWER 5 OF 12 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1986:605107 CAPLUS
DOCUMENT NUMBER: 105:205107
TITLE: Renal plasma membrane receptors for certain modified serum albumins. Evidence for participation of a heparin receptor
AUTHOR(S): Ranganathan, Perungavur N.; Mego, John L.
CORPORATE SOURCE: Biol. Dep., Univ. Alabama, University, AL, 35486, USA
SOURCE: Biochemical Journal (1986), 239(3), 537-43
CODEN: BIJOAK; ISSN: 0306-3275
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The binding of formaldehyde-treated (f-alb), reduced-carboxymethylated (ac-alb) or reduced-acetamidated (am-alb) bovine serum albumins to purified rat renal plasma membranes was studied. Radioiodinated f-alb or ac-alb bound to kidney membranes, whereas am-alb neither bound significantly nor competed with f-alb binding to kidney membranes. The binding was specific, saturable, and heat- and proteinase -sensitive. Competition studies showed that f-alb and ac-alb sites may be the same on these membranes. To determine the role played by charge in binding, competition expts. with polyanions were performed. Polyanions, such as nucleic acid or glycosaminoglycans, were

effective competitors of f-alb binding to cell membranes. Heparin was especially inhibitory, being several-fold more so than chondroitin sulfate. Completely reduced and carboxymethylated albumin was a better competitor than its partially modified counterpart. Furthermore, f-alb was a significant competitor of [35S]heparin binding to kidney membranes. Also, partially purified heparin receptor demonstrated specific binding of 125I-f-alb. The data suggested that a heparin receptor is responsible for binding and internalization of i.v. injected f-alb. A Scatchard plot revealed 2 classes of receptors with dissociation consts. of 3.2 + 10-6 and 4.7 + 10-5 M.

L3 ANSWER 6 OF 12 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1985:558422 CAPLUS
DOCUMENT NUMBER: 103:158422
TITLE: Basic protein-glycosaminoglycan interaction as a possible primary cause of vascular and cardiac damage
AUTHOR(S): Tedeschi, Guido G.; Cingolani, Rosalia Tacconi
CORPORATE SOURCE: Sch. Clin. Chem. Bacteriol., Univ. Camerino, Camerino, 62032, Italy
SOURCE: IRCS Medical Science (1985), 13(6), 514
CODEN: IMSCE2; ISSN: 0268-8220
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The addition of hyaluronic acid and/or chondroitin sulfate to histone or human globin solns. caused the immediate formation of voluminous aggregates of eosinophil particles and fibrouis material with strong affinity towards Alcian blue, insol. in the pH range 2-13. The addition of hyaluronic acid and/or chondroitin sulfate solns. to β-hemolytic Streptococcus group A cultures from the beginning of the incubation or at various time intervals, caused rapid agglutination and sedimentation, lysis, and the appearance of granular material which condensed in large aggregates held together by fibrous structures embedding the bacterial bodies. A similar addition to the 4500 + g supernatant gave rise to a precipitate containing a small number of altered bacteria and a relatively large amount of low mol. weight nucleic acids. No nucleic acids in a detectable amount were present in the precipitate produced following addition of hyaluronic acid and/or chondroitin sulfate to the 0.45 μm filtrate of the cultures. The expts. where saline solns. of purified histone proteins and glycosaminoglycans were used indicate that the precipitation may be attributed to the presence of basic proteins within the cultures. The initial formation of particulate material in vivo accompanied by deposit at the cardiac and vascular level, could be attributed to interaction between basic proteins and glycosaminoglycans, independent of the presence of lipid components. This process could be enhanced during the course of inflammation, especially if it was caused or accompanied by bacterial growth.

L3 ANSWER 7 OF 12 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1969:487745 CAPLUS
DOCUMENT NUMBER: 71:87745
TITLE: Protein-polysaccharides from pig laryngeal cartilage. Extraction and purification
AUTHOR(S): Tsiganos, Constantine P.; Muir, Helen
CORPORATE SOURCE: Kennedy Inst. Rheumatol., London, UK
SOURCE: Biochemical Journal (1969), 113(5), 879-84
CODEN: BIJOAK; ISSN: 0264-6021
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Protein-polysaccharides of chondroitin sulfate were extracted from fresh laryngeal cartilage at pH 6.8 by 2 procedures. Procedure 1 consisted of brief low-speed homogenization in 0.15M (iso-osmotic) NaOAc and procedure 2 consisted of longer homogenization followed by prolonged

extraction in 10% CaCl2 solution The protein-polysaccharides in both exts. were isolated and purified by precipitation with 9-aminoacridine-HCl. They were free from serum proteins, collagen, and nucleic acids and also of degradative enzymes. The absence of such enzymes was shown by viscosity measurements on solns. of protein-polysaccharides incubated for up to 24 hrs. at pH 4 and 6.8. Mannose, glucose, or fucose was not detected by paper chromatog. and only traces of sialic acid were present. The yield with procedure 2 was twice that with procedure 1 and the products differed in their protein and glucosamine contents. Hyaluronic acid was unlikely to have been precipitated at an acid pH, so the glucosamine was attributed to keratan sulfate, as serum proteins were absent. There was no free keratan sulfate in the preparation Both prepns. were heterogeneous in the ultracentrifuge, showing at least 3 components.

L3 ANSWER 8 OF 12 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1969:8827 CAPLUS
DOCUMENT NUMBER: 70:8827
TITLE: Purification and characterization of sulfated glycoproteins and hyaluronidase-resistant mucopolysaccharides from dog gastric mucosa
AUTHOR(S): Pamer, Treva; Glass, George B.; Horowitz, Martin I.
CORPORATE SOURCE: New York Med. Coll., New York, NY, USA
SOURCE: Biochemistry (1968), 7(11), 3821-9
CODEN: BICHAW; ISSN: 0006-2960
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Sulfated glycoproteins were purified from papain-digested mucosal scrapings of the dog fundic stomach. The acidic fraction, isolated by EtOH and cetylpyridinium chloride pptns., was freed of nucleic acids by precipitation at pH 1.5 and of digestible mucopolysaccharides by treatment with testicular hyaluronidase. After gel filtration on Bio-Gel P-30 and Sephadex G-100, final separation of three sulfated glycoprotein fractions was achieved by elution from DEAE-Sephadex with NaCl. Each fraction exhibited a single band on cellulose acetate electrophoresis at pH 3 and 9. The 3 fractions were similar in having equimolar ratios of hexosamine and galactose, the major carbohydrate components, and in having glucosamine and galactosamine in equimolar ratios. SO42- was present in each fraction. Fucose and sialic acid content decreased with increasing molarity of eluting salt. Total protein ranged from 10.9 to 12%. Threonine, serine, proline, and valine constituted 88% of the total amino acid residues while aromatic and S amino acids were present only in trace quantities. Two hyaluronidase-resistant mucopolysaccharides were partially purified by Cu precipitation and tentatively identified as chondroitin sulfate-B and heparitin sulfate on the basis of their reactions in the carbazole and orcinol assays for uronic acids.

L3 ANSWER 9 OF 12 MEDLINE on STN
ACCESSION NUMBER: 93289951 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8512063
TITLE: Simultaneous preparation and quantitation of proteoglycans by precipitation with alcian blue.
AUTHOR: Bjornsson S
CORPORATE SOURCE: Department of Clinical Chemistry, University Hospital, Lund, Sweden.
SOURCE: Analytical biochemistry, (1993 May 1) Vol. 210, No. 2, pp. 282-91.
Journal code: 0370535. ISSN: 0003-2697.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199307

ENTRY DATE: Entered STN: 23 Jul 1993
Last Updated on STN: 6 Feb 1998
Entered Medline: 9 Jul 1993

AB Conditions for specific interaction between Alcian blue and proteoglycans were optimized by comparing the differential spectra of Alcian blue obtained with purified chondroitin sulfate dissolved in water with the spectra obtained with nasal cartilage proteoglycans dissolved in synovial fluid. A method was then designed that provides specific precipitation of proteoglycans or glycosaminoglycans in 4 M guanidine - HCl in the presence of protein, hyaluronic acid, or nucleic acids. The specificity is achieved by using a low pH in combination with detergent and high salt concentration. Stepwise addition of reagents is necessary to avoid binding of Alcian blue to proteins and nucleic acids. All polyanions, except polysulfates, are first neutralized by lowering the pH to 1.5. By including detergent in this step, the hydrophobic protein regions are blocked and not accessible for binding with the dye. These regions could otherwise bind Alcian blue by hydrophobic interaction. When the Alcian blue reagent is added after, only the polysulfated molecules will remain charged and free to interact with Alcian blue. At least 0.4 M guanidine-HCl is required to abolish the negative interference by proteins. All sulfated glycosaminoglycans are precipitated at 0.4 M guanidine-HCl. With increasing guanidine-HCl concentrations, the different glycosaminoglycans are precipitated in accordance with the critical electrolyte concentration of the respective glycosaminoglycan. The Alcian blue precipitation can be performed at different concentrations of guanidine-HCl in order to separate different classes of proteoglycans. Excess dye and contaminating proteins are removed by a wash in a DMSO-MgCl2 solution and the precipitate is dissolved in a mixture of guanidine-HCl and propanol. For quantitation, the absorbance is recorded in a microplate reader with the 600-nm filter, the assay being linear between 0.5 and 20 micrograms proteoglycan. Since no digestion of samples with protease is needed, the proteoglycans are recovered in native form. The proteoglycan-Alcian blue complexes dissociate in the guanidine-HCl/propanol mixture and the proteoglycans can be selectively precipitated with propanol. The dye is used for quantitation and the proteoglycans can be utilized for further analysis.

L3 ANSWER 10 OF 12 MEDLINE on STN
ACCESSION NUMBER: 92175078 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1541329
TITLE: High molecular weight mucin-like glycoproteins of the bovine interphotoreceptor matrix.
AUTHOR: Plantner J J
CORPORATE SOURCE: Lorand V. Johnson Laboratory for Research in Ophthalmology, Department of Surgery, Case Western Reserve University, Cleveland, OH 44106.
CONTRACT NUMBER: EY 06571 (NEI)
SOURCE: Experimental eye research, (1992 Jan) Vol. 54, No. 1, pp. 113-25.
Journal code: 0370707. ISSN: 0014-4835.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199204
ENTRY DATE: Entered STN: 24 Apr 1992
Last Updated on STN: 24 Apr 1992
Entered Medline: 8 Apr 1992

AB A very high molecular weight mucin-like glycoprotein was isolated by gel filtration of interphotoreceptor matrix (IPM) from fresh bovine eyes and purified to apparent homogeneity by cesium chloride/guanidine hydrochloride (GuHCl) equilibrium density gradient centrifugation.

Although a molecular weight in excess of 10(7) Da is suggested by gel filtration, the presence of SDS or GuHCl did not alter its elution position, indicating that the large size was not simply due to aggregation. Treatment of this material with disulfide reagents, however, led to a decrease in molecular size. On a relative basis, substantially more of this glycoprotein is present in IPM prepared from retina than from retinal pigment epithelium. While the carbohydrate and amino acid composition are not those of a true 'mucin', the large size and many other properties are quite 'mucin-like'. The carbohydrate composition suggests the presence of both N- and O-glycosidically linked sugar chains. The presence of a mucin-type O-glycosidic linkage is indicated by its susceptibility to alkaline cleavage, with concomitant loss of serine and threonine and increase in 240 nm absorbance; production of a fluorescent product upon reaction with cyanoacetamide; lectin binding properties; and production of N-acetylgalactosaminitol upon alkaline borohydride elimination. This glycoprotein was digested by pronase and trypsin, confirming its protein nature, but was resistant to digestion with chondroitin ABC lyase, hyaluronidase and heparinase, as well as RNAase, indicating that these components were not present to any appreciable extent. ELISA for cartilage keratan sulfate was also negative. Centrifugation in CsCl/GuHCl gradients indicated a density much lower than that of a proteoglycan or nucleic acid as well. In vitro biosynthetic studies suggest that both retina and retinal pigment epithelium may be major sources of material in the IPM. The elution patterns of radioactivity were strikingly similar to the UV elution patterns of IPM. The medium from retinal incubations contained very high molecular weight material which was resistant to enzymes which hydrolyse glycosaminoglycans, suggesting that retina may be the source of this high molecular weight, mucin-like glycoprotein.

L3 ANSWER 11 OF 12 MEDLINE on STN
ACCESSION NUMBER: 88207935 MEDLINE
DOCUMENT NUMBER: PubMed ID: 3129962
TITLE: Densitometric assay of nanogram quantities of proteoglycans precipitated on nitrocellulose membrane with Safranin O.
AUTHOR: Lammi M; Tammi M
CORPORATE SOURCE: Department of Anatomy, University of Kuopio, Finland.
SOURCE: Analytical biochemistry, (1988 Feb 1) Vol. 168, No. 2, pp. 352-7.
Journal code: 0370535. ISSN: 0003-2697.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198805
ENTRY DATE: Entered STN: 8 Mar 1990
Last Updated on STN: 8 Mar 1990
Entered Medline: 31 May 1988

AB Proteoglycan (PG) and glycosaminoglycan (GAG) samples corresponding to a minimum of 10 ng of uronic acid were reliably quantified as precipitates with the cationic dye Safranin O, collected by vacuum-aided filtration onto a cellulose acetate/nitrate membrane in a standard 96-well dot assay apparatus. The reflectances of the precipitation dots were measured by automatic densitometric scanning of the membrane sheets. Standard GAGs produced reflectance values which were related to the number of anionic groups per unit disaccharide; hyaluronate and keratan sulfate gave lower values while heparin yielded values higher than those of chondroitin sulfates. The presence of 8 M urea, 1% Triton X-100, 30% sucrose, 0.02% NaN3, or mixtures of proteinase inhibitors and various buffers did not markedly influence the reflectances, while 4 M guanidinium chloride and 3 M CsCl reduced the sensitivity of the assay to 30-50 ng. Samples containing sodium dodecyl sulfate (SDS) were not applicable because SDS precipitated with Safranin O. Proteins showed virtually no response, while nucleic acids gave

significant although smaller reflectances than GAGs. Owing to its marked sensitivity and convenience the method is particularly suitable for the detection of PGs during their preparative purification and fractionation as well as in various analytical assays.

L3 ANSWER 12 OF 12 MEDLINE on STN
ACCESSION NUMBER: 87156514 MEDLINE
DOCUMENT NUMBER: PubMed ID: 3030264
TITLE: Renal plasma membrane receptors for certain modified serum albumins. Evidence for participation of a heparin receptor.
AUTHOR: Ranganathan P N; Mego J L
SOURCE: The Biochemical journal, (1986 Nov 1) Vol. 239, No. 3, pp. 537-43.
Journal code: 2984726R. ISSN: 0264-6021.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198704
ENTRY DATE: Entered STN: 3 Mar 1990
Last Updated on STN: 3 Mar 1990
Entered Medline: 1 Apr 1987

AB Binding of formaldehyde-treated (f-alb), reduced-carboxymethylated (ac-alb) or reduced-acetamidated (am-alb) bovine serum albumins to purified rat renal plasma membranes was studied. Radioiodinated f-alb or ac-alb bound to kidney membranes while am-alb neither bound significantly nor competed with f-alb binding to kidney membranes. The binding was specific, saturable and heat- and proteinase -sensitive. Competition studies showed that f-alb and ac-alb sites may be the same on these membranes. To determine the role played by charge in binding, competition experiments with polyanions were performed. Polyanions such as nucleic acid or glycosaminoglycans were effective competitors of f-alb binding to cell membranes. Heparin was especially inhibitory, being several-fold more so than chondroitin sulphate. Completely reduced and carboxymethylated albumin was a better competitor than its partially modified counterpart. Furthermore, f-alb was a significant competitor of [35S]heparin binding to kidney membranes. Also, partially purified heparin receptor demonstrated specific binding of 125I-f-alb. These data suggest that a heparin receptor is responsible for binding and internalization of intravenously injected f-alb. A Scatchard plot revealed two classes of receptors with dissociation constants of 3.2 X 10(-6) M and 4.7 X 10(-5) M.

L5 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1987:107899 CAPLUS
DOCUMENT NUMBER: 106:107899
TITLE: Isolation of heparan sulfate and dermatan sulfate from arteries and cardiac muscles
PATENT ASSIGNEE(S): Mediolanum Farmaceutici S.r.l., Italy
SOURCE: Jpn. Kokai Tokkyo Koho, 8 pp.
CODEN: JKXXAF
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| JP 61218601 | A2 | 19860929 | JP 1986-53838 | 19860313 |
| JP 02038601 | B4 | 19900831 | | |
| EP 199033 | A2 | 19861029 | EP 1986-102963 | 19860306 |
| EP 199033 | A3 | 19880330 | | |
| EP 199033 | B1 | 19940105 | | |
| R: AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE | | | | |
| AT 99702 | E | 19940115 | AT 1986-102963 | 19860306 |
| US 4783447 | A | 19881108 | US 1986-838133 | 19860310 |
| DK 8601138 | A | 19860914 | DK 1986-1138 | 19860312 |
| FI 8601020 | A | 19860914 | FI 1986-1020 | 19860312 |
| FI 82700 | B | 19901231 | | |
| FI 82700 | C | 19910410 | | |
| NO 8600940 | A | 19860915 | NO 1986-940 | 19860312 |
| NO 166187 | B | 19910304 | | |
| NO 166187 | C | 19910612 | | |
| ZA 8601855 | A | 19861029 | ZA 1986-1855 | 19860312 |
| ES 552898 | A1 | 19870516 | ES 1986-552898 | 19860312 |
| IN 163616 | A | 19881015 | IN 1986-CA184 | 19860312 |
| CA 1286286 | A1 | 19910716 | CA 1986-503943 | 19860312 |
| AU 8654727 | A1 | 19860918 | AU 1986-54727 | 19860313 |
| AU 582221 | B2 | 19890316 | | |

PRIORITY APPLN. INFO.: IT 1985-19885 A 19850313
EP 1986-102963 A 19860306

AB Heparan sulfate and dermatan sulfate for treatment of venous thrombosis are isolated in pure form from arteries and cardiac muscles. The method comprises (1) extraction of proteoglycans from the tissues using urea in the extraction solution, (2) the solution is filtered, and urea eliminated, (3) mucopolysaccharides are separated from proteins, (4) proteins precipitated and filtered out, (5) nucleic acids eliminated, (6) mucopolysaccharides precipitated, and (7) heparan sulfate and dermatan sulfate extracted and purified. From 1000 kg of arteries and cardiac muscles of mammals, 240 g heparan sulfate and 150 g dermatan sulfate were obtained.

L8 ANSWER 1 OF 3 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2005:61554 CAPLUS
DOCUMENT NUMBER: 142:293602
TITLE: Extracellular RNA is a natural cofactor for the (auto-)activation of Factor VII-activating protease (FSAP)
AUTHOR(S): Nakazawa, Fumie; Kannemeier, Christian; Shibamiya, Aya; Song, Yutong; Tzima, Eleni; Schubert, Uwe; Koyama, Takatoshi; Niepmann, Michael; Trusheim, Heidi; Engelmann, Bernd; Preissner, Klaus T.
CORPORATE SOURCE: Graduate School of Allied Health Sciences, Tokyo Medical and Dental University, Bunkyo-ku, Tokyo, 113-8519, Japan
SOURCE: Biochemical Journal (2005), 385(3), 831-838
CODEN: BIJOAK; ISSN: 0264-6021
PUBLISHER: Portland Press Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB FSAP (Factor VII-activating protease) is a new plasma-derived serine protease with putative dual functions in hemostasis, including activation of coagulation Factor VII and generation of urinary-type plasminogen activator (urokinase). The (auto-)activation of FSAP is facilitated by polyanionic glycosaminoglycans, such as heparin or dextran sulfate, whereas calcium ions stabilize the active form of FSAP. In the present study, extracellular RNA was identified and characterized as a novel FSAP cofactor. The conditioned medium derived from various cell types such as smooth muscle cells, endothelial cells, osteosarcoma cells or CHO (Chinese-hamster ovary) cells contained an acidic factor that initiated (auto-)activation of FSAP. RNase A, but not other hydrolytic enzymes (proteases, glycanases and DNase), abolished the FSAP cofactor activity, which was subsequently isolated by anion-exchange chromatog. and unequivocally identified as RNA. In purified systems, as well as in plasma, different forms of natural RNA (rRNA, tRNA, viral RNA and artificial RNA) were able to (auto-)activate FSAP into the two-chain enzyme form. The specific binding of FSAP to RNA (but not to DNA) was shown by mobility-shift assays and UV crosslinking, thereby identifying FSAP as a new extracellular RNA-binding protein, the KD estimated to be 170-350 nM. Activation of FSAP occurred through an RNA-dependent template mechanism involving a nucleic acid size of at least 100 nt. In a purified system, natural RNA augmented the FSAP-dependent Factor VII activation several-fold (as shown by subsequent Factor Xa generation), as well as the FSAP-mediated generation of urokinase. The results provide evidence for the first time that extracellular RNA, present at sites of cell damage or vascular injury, can serve an important as yet unrecognized cofactor function in hemostasis by inducing (auto-)activation of FSAP through a novel surface-dependent mechanism.
REFERENCE COUNT: 21 THERE ARE 21 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 2 OF 3 MEDLINE on STN
ACCESSION NUMBER: 2000095859 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10631998
TITLE: Mass spectral characterization of a protein-nucleic acid photocrosslink.
AUTHOR: Golden M C; Resing K A; Collins B D; Willis M C; Koch T H
CORPORATE SOURCE: Department of Chemistry and Biochemistry, University of Colorado, Boulder 80309-0215, USA.
CONTRACT NUMBER: AR43768 (NIAMS)
SOURCE: Protein science : a publication of the Protein Society, (1999 Dec) Vol. 8, No. 12, pp. 2806-12.
Journal code: 9211750. ISSN: 0961-8368.
PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200002
ENTRY DATE: Entered STN: 29 Feb 2000
Last Updated on STN: 29 Feb 2000
Entered Medline: 14 Feb 2000

AB A photocrosslink between basic fibroblast growth factor (bFGF155) and a high affinity ssDNA oligonucleotide was characterized by positive ion electrospray ionization mass spectrometry (ESIMS). The DNA was a 61-mer oligonucleotide photoaptamer bearing seven bromodeoxyuridines, identified by in vitro selection. Specific photocrosslinking of the protein to the oligonucleotide was achieved by 308 nm XeCl excimer laser excitation. The cross-linked protein nucleic acid complex was proteolyzed with trypsin. The resulting peptide crosslink was purified by PAGE, eluted, and digested by snake venom phosphodiesterase/alkaline phosphatase. Comparison of the oligonucleotide vs. the degraded peptide crosslink by high performance liquid chromatography coupled to an electrospray ionization triple quadrupole mass spectrometer showed a single ion unique to the crosslinked material. Sequencing by collision induced dissociation (MS/MS) on a triple quadrupole mass spectrometer revealed that this ion was the nonapeptide TGQYKLGSK (residues 130-138) crosslinked to a dinucleotide at Tyr133. The MS/MS spectrum indicated sequential fragmentation of the oligonucleotide to uracil covalently attached to the nonapeptide followed by fragmentation of the peptide bonds. Tyr133 is located within the heparin binding pocket, suggesting that the in vitro selection targeted this negative ion binding region of bFGF155.

L8 ANSWER 3 OF 3 MEDLINE on STN
ACCESSION NUMBER: 92348600 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1379251
TITLE: Purification, characterization and crystallization of recombinant HIV-1 reverse transcriptase.
AUTHOR: Bhikhabhai R; Joelson T; Unge T; Strandberg B; Carlsson T; Lovgren S
CORPORATE SOURCE: Department of Molecular Biology, Uppsala University, Sweden.
SOURCE: Journal of chromatography, (1992 Jun 26) Vol. 604, No. 1, pp. 157-70.
Journal code: 0427043. ISSN: 0021-9673.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; AIDS
ENTRY MONTH: 199209
ENTRY DATE: Entered STN: 11 Sep 1992
Last Updated on STN: 3 Feb 1997
Entered Medline: 3 Sep 1992

AB The pol I gene from HIV-1 encoding the protease, reverse transcriptase (RT) and endonuclease has been expressed in Escherichia coli. By modifying the fermentation conditions and developing a new purification scheme, the yield of purified RT has been increased substantially compared with that obtained in an earlier procedure. The expressed RT was purified to homogeneity by ammonium sulphate fractionation followed by chromatography on DEAE Sepharose, Heparin Sepharose, S Sepharose and Poly(A)-Sepharose. The purified HIV-RT is a heterodimer (p66/p51) with an isoelectric point close to 8 and with a tendency to aggregate. The proteolytic product (p51), corresponding to the N-terminal end of the RT molecule, was isolated and identified, as were also some bacterial polypeptides that co-elute with HIV-RT during the early stages of the purification. The heterodimer was crystallized in several morphological forms using the vapour-diffusion hanging drop technique. To

concentrate the protein and to change the buffer for crystallization, reverse-salt-gradient chromatography and micropreparative columns were used. The best crystals diffracted to 9 A resolution. The best crystals of native RT diffracted to 9 A resolution and in complex with nucleic acids to 4.5 A resolution (using a rotating anode X-ray source).

L11 ANSWER 1 OF 8 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2003:760679 CAPLUS
DOCUMENT NUMBER: 140:24627
TITLE: Adaptor protein Ruk1 forms protein-protein complexes with endonuclease activity in HEK293 cells
AUTHOR(S): Kit, Yu. Ya.; Drel, V. R.; Petriv, O. I.; Kovalyova, V. A.; Shuvaeva, G. Yu.; Palivoda, O. Yu.; Vovk, E. I.; Bobak, Ya. P.; Rzeszowska-Wolny, J.; Gout, I. T.; Buchman, V. L.; Drobot, L. B.
CORPORATE SOURCE: Institute of Cell Biology, National Academy of Sciences of Ukraine, Lvov, 79005, Ukraine
SOURCE: Biochemistry (Moscow, Russian Federation)(Translation of Biokhimiya (Moscow, Russian Federation)) (2003), 68(7), 810-815
CODEN: BIORAK; ISSN: 0006-2979
PUBLISHER: MAIK Nauka/Interperiodica Publishing
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The structural and functional organization of the adaptor protein Ruk1 is characterized by the presence of three SH3-domains at the N-terminus followed by Pro- and Ser-rich sequences and a C-terminal coiled-coil region. Multiple modules in the Ruk1 structure involved in protein-protein interactions can provide for formation of ligand clusters with varied properties and subcellular location. To study the nature and biol. role of such complexes, the recombinant protein Ruk1 with a Glu-epitope at the C-terminus (Ruk1 Glu-tagged) was purified from transfected HEK293 cells by affinity chromatog. on protein G-Sepharose with covalently conjugated anti-Glu-tag antibodies. By SDS-PAGE with subsequent staining with silver, a set of minor bands in addition to the 85 kDa Ruk1 Glu-tagged was detected in the purified preparation of the recombinant protein. Proteins with affinity for nucleic acids were also revealed in the Ruk1 Glu-tagged preparation by retardation of electrophoretic mobility of 32P-labeled oligodeoxyribonucleotides in gel. The Ruk1 Glu-tagged preparation was also shown to hydrolyze both deoxyribonucleotides and plasmid DNA. ZnCl2 and heparin inhibited the DNAse activity. These findings suggest the presence of DNases associated with the Ruk1 protein in HEK293 cells. Such complexes were isolated from lysates of HEK293 cells by chromatog. on heparin-Sepharose. By elution with 0.5 and 1.0 M NaCl, two fractions with DNase activity and containing proteins with mol. wts. of 83, 80 and 72 kDa were obtained. The reaction was inhibited by ZnCl2 and heparin, and previous precipitation of Ruk-related proteins with anti-Ruk antibodies resulted in the exhaustion of nuclease activity. By immunoblotting with anti-Ruk antibodies, 83 kDa protein immunol. related to the Ruk1 protein was identified in the fractions. It was concluded that the adaptor protein Ruk1 forms complexes with endonucleases in HEK293 cells.
REFERENCE COUNT: 22 THERE ARE 22 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L11 ANSWER 2 OF 8 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1990:419882 CAPLUS
DOCUMENT NUMBER: 113:19882
TITLE: An inducible 3'-nucleotidase/nuclease from the trypanosomatid Crithidia luciliae. Purification and characterization
AUTHOR(S): Neubert, Thomas A.; Gottlieb, Michael
CORPORATE SOURCE: Sch. Hyg. Public Health, Johns Hopkins Univ., Baltimore, MD, 21205, USA
SOURCE: Journal of Biological Chemistry (1990), 265(13), 7236-42

CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Several species of protozoan parasites of the family Trypanosomatidae have a surface membrane-associated enzyme which is capable of hydrolyzing extracellular 3'-nucleotides and nucleic acids, thereby aiding in the acquisition of nutritionally required purines and phosphate (Pi) from their hosts. In C. luciliae, this 3'-nucleotidase/nuclease previously has been shown to be highly regulated as purine and/or Pi starvation of this trypanosomatid leads to as much as a 1000-fold increase in enzyme activity. The enzyme was purified to apparent homogeneity from detergent exts. of purine-starved C. luciliae by heparin-agarose chromatog. followed by Mono Q and Mono S fast protein liquid chromatog. The enzyme had an apparent mol. wt. of 43,000 and a pI of .apprx.5.8. The enzyme displayed broad pH optima, with peaks at 8.0, for both nucleotidase and nuclease activities. The pH optima shifted to lower values when the activity was assayed in the presence of sulfhydryl reagents. The enzyme was most active with 3'-AMP and poly(A) in nucleotidase and nuclease assays, resp. As a nuclease the enzyme hydrolyzed RNA at a faster rate than single-standard DNA with no detectable hydrolysis of double-standard DNA. The loss of enzyme activity which occurred upon storage at acid pH was prevented by the inclusion of Zn2+ in storage buffers. The physicochem. and kinetic properties of this trypanosomatid enzyme suggest that it is similar to the class I nucleases found in fungi and in germinating seedlings of higher plants.

L11 ANSWER 3 OF 8 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1978:542455 CAPLUS
DOCUMENT NUMBER: 89:142455
TITLE: Enzymochemical studies on snake venoms. III. Purification and properties of arginine esterase which possesses clotting activity in the venom of Agkistrodon acutus
AUTHOR(S): Sugihara, Hisayoshi; Nikai, Toshiaki; Oda, Humihiko; Tanaka, Tetsunosuke
CORPORATE SOURCE: Fac. Pharm., Meijo Univ., Nagoya, Japan
SOURCE: Yakugaku Zasshi (1978), 98(7), 832-9
CODEN: YKKZAJ; ISSN: 0031-6903
DOCUMENT TYPE: Journal
LANGUAGE: Japanese
AB A human blood-coagulation principle was isolated from the venom of the snake A. acutus by a combination of gel filtration on Sephadex G 75 and chromatog. on DEAE-Sephadex A 50, CM-Sephadex C 50, and DEAE-Sephadex A 50. A 17.4-mg purified preparation was obtained from 1 g of crude venom. This coagulation principle hydrolyzed arginine esters, such as tosyl-L-arginine Me ester or benzoylarginine Et ester, but did not digest casein. The coagulation and esterolytic activities were inhibited by diisopropylfluorophosphate or antiserum but not by soybean trypsin inhibitor, EDTA, cysteine, heparin, or p-chloromercuribenzoate. The preparation was homogeneous as judged by disc electrophoresis on polyacrylamide gel and isoelec. focusing. The mol. wt. was .apprx.52,000, and the isoelec. point was pH 4.7. The coagulation and esterolytic activities of this protein were 37.5 and 99.3 units, resp. This protein was stable to heat treatment and pH values of 6-8. Michaelis constant (Km) and inhibition constant (Ki) for this protein were 1.34 + 10-3M and 1.92 + 10-3M, resp. This enzyme contained some carbohydrate but did not contain any nucleic acids.

L11 ANSWER 4 OF 8 MEDLINE on STN
ACCESSION NUMBER: 2003407723 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12946264
TITLE: Adaptor protein Ruk1 forms protein-protein complexes with

endonuclease activity in HEK293 cells.
AUTHOR: Kit Yu Ya; Drel V R; Petriv O I; Kovalyova V A; Shuvaeva G Yu; Palivoda O Yu; Vovk E I; Bobak Ya P; Rzeszowska-Wolny J; Gout I T; Buchman V L; Drobot L B
CORPORATE SOURCE: Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv, 79005, Ukraine.
SOURCE: Biochemistry. Biokhimii a, (2003 Jul) Vol. 68, No. 7, pp. 810-5.
Journal code: 0376536. ISSN: 0006-2979.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200405
ENTRY DATE: Entered STN: 30 Aug 2003
Last Updated on STN: 20 May 2004
Entered Medline: 19 May 2004

AB The structural and functional organization of the adaptor protein Ruk(1) is characterized by the presence of three SH3-domains at the N-terminus followed by Pro- and Ser-rich sequences and a C-terminal coiled-coil region. Multiple modules in the Ruk(1) structure involved in protein-protein interactions can provide for formation of ligand clusters with varied properties and subcellular location. To study the nature and biological role of such complexes, the recombinant protein Ruk(1) with a Glu-epitope at the C-terminus (Ruk(1) Glu-tagged) was purified from transfected HEK293 cells by affinity chromatography on protein G-Sepharose with covalently conjugated anti-Glu-tag antibodies. By SDS polyacrylamide gel electrophoresis with subsequent staining with silver, a set of minor bands in addition to the 85-kD Ruk(1) Glu-tagged was detected in the purified preparation of the recombinant protein. Proteins with affinity for nucleic acids were also revealed in the Ruk(1) Glu-tagged preparation by retardation of electrophoretic mobility of 32P-labeled oligodeoxyribonucleotides in gel. The Ruk(1) Glu-tagged preparation was also shown to hydrolyze both deoxyribonucleotides and plasmid DNA. ZnCl2 and heparin inhibited the DNAse activity. These findings suggest the presence of DNases associated with the Ruk(1) protein in HEK293 cells. Such complexes were isolated from lysates of HEK293 cells by chromatography on heparin-Sepharose. By elution with 0.5 and 1.0 M NaCl, two fractions with DNase activity and containing proteins with molecular weights of 83, 80, and 72 kD were obtained. The reaction was inhibited by ZnCl2 and heparin, and previous precipitation of Ruk-related proteins with anti-Ruk antibodies resulted in the exhaustion of nuclease activity. By immunoblotting with anti-Ruk antibodies, 83-kD protein immunologically related to the Ruk(1) protein was identified in the fractions. It was concluded that the adaptor protein Ruk(1) forms complexes with endonucleases in HEK293 cells.

L11 ANSWER 5 OF 8 MEDLINE on STN
ACCESSION NUMBER: 97234632 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9079906
TITLE: Purification and characterization of Mycoplasma penetrans Ca2+/Mg2+-dependent endonuclease.
AUTHOR: Bendjennat M; Blanchard A; Loutfi M; Montagnier L; Bahraoui E
CORPORATE SOURCE: Laboratory of Immunovirology UFR SVT, University of Paul Sabatier, Toulouse, France.
SOURCE: Journal of bacteriology, (1997 Apr) Vol. 179, No. 7, pp. 2210-20.
Journal code: 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English
FILE SEGMENT: Priority Journals; AIDS
ENTRY MONTH: 199704
ENTRY DATE: Entered STN: 7 May 1997
Last Updated on STN: 3 Mar 2000
Entered Medline: 29 Apr 1997

AB The major nuclease from Mycoplasma penetrans has been purified to homogeneity. The enzyme seems to be present as a membrane-associated precursor of 50 kDa and as a peripheral membrane monomeric polypeptide of 40 kDa that is easily removed by washing of cells with isotonic buffers and in the aqueous phase upon Triton partitioning of Triton X-114-solubilized protein. The 40-kDa nuclease was extracted from M. penetrans cells by Triton X-114 and phase fractionation and was further purified by chromatography on Superdex 75 and chelating Sepharose (Zn2+ form) columns. By gel filtration, the apparent molecular mass was 40 kDa. The purified enzyme exhibits both a nicking activity on superhelical and linear double-stranded DNA and a nuclease activity on RNA and single-stranded DNA. No exonuclease activity was found for this enzyme. This nuclease required both Mg2+ (optimum, 5 mM) and Ca2+ (optimum, 2 mM) for activity and exhibited a pH optimum between pH 7 and 8 for DNase activity. It was inhibited by Zn2+, Mn2+, heparin, sodium dodecyl sulfate, and chelator agents such EDTA and EGTA, but no effect was observed with ATP, 2-mercaptoethanol, N-ethylmaleimide, dithiothreitol, nonionic detergents, phenylmethylsulfonyl fluoride, and iodoacetamide. Nuclease activity was inhibited by diethylpyrocarbonate at both pH 6 and 8 and by pepstatin, suggesting the involvement of a histidine and an aspartate in the active site. When added to human lymphoblast nuclei, the purified M. penetrans endonuclease induced internucleosomal fragmentation of the chomatin into oligonucleosomal fragments. On the basis of this result, and taking into account the fact that M. penetrans has the capacity to invade eucaryotic cells, one can suggest, but not assert, that produced Ca2+/Mg2+-dependent endonuclease may alter the nucleic acid metabolism of host cells by DNA and/or RNA degradation and may act as a potential pathogenic determinant.

L11 ANSWER 6 OF 8 MEDLINE on STN
ACCESSION NUMBER: 90237014 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2158995
TITLE: An inducible 3'-nucleotidase/nuclease from the trypanosomatid Crithidia luciliae. Purification and characterization.
AUTHOR: Neubert T A; Gottlieb M
CORPORATE SOURCE: Department of Immunology and Infectious Diseases, Johns Hopkins University School of Hygiene and Public Health, Baltimore, Maryland 21205.
CONTRACT NUMBER: AI-16530 (NIAID)
SOURCE: The Journal of biological chemistry, (1990 May 5) Vol. 265, No. 13, pp. 7236-42.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199006
ENTRY DATE: Entered STN: 6 Jul 1990
Last Updated on STN: 3 Feb 1997
Entered Medline: 4 Jun 1990

AB Several species of protozoan parasites of the family Trypanosomatidae have a surface membrane-associated enzyme which is capable of hydrolyzing extracellular 3'-nucleotides and nucleic acids, thereby aiding in the acquisition of nutritionally required purines and Pi from their hosts. In Crithidia luciliae, this 3'-nucleotidase/nuclease previously has been shown to be highly regulated as purine and/or Pi

starvation of this trypanosomatid leads to as much as a 1000-fold increase in enzyme activity. We have purified the enzyme to apparent homogeneity from detergent extracts of purine-starved C. luciliae by heparin-agarose chromatography followed by Mono Q and Mono S fast protein liquid chromatography. The enzyme had an apparent molecular weight of 43,000 and a pI of approximately 5.8. The enzyme displayed broad pH optima, with peaks at 8.0, for both nucleotidase and nuclease activities. The pH optima shifted to lower values when the activity was assayed in the presence of sulfhydryl reagents. The enzyme was most active with 3'-AMP and poly(A) in nucleotidase and nuclease assays, respectively. As a nuclease the enzyme hydrolyzed RNA at a faster rate than single-stranded DNA with no detectable hydrolysis of double-stranded DNA. The loss of enzyme activity which occurred upon storage at acid pH was prevented by the inclusion of Zn2+ in storage buffers. The physicochemical and kinetic properties of this trypanosomatid enzyme suggest that it is similar to the class I nucleases found in fungi and in germinating seedlings of higher plants.

L11 ANSWER 7 OF 8 MEDLINE on STN
ACCESSION NUMBER: 88196886 MEDLINE
DOCUMENT NUMBER: PubMed ID: 3129338
TITLE: Immunopurification of heterogeneous nuclear ribonucleoprotein particles reveals an assortment of RNA-binding proteins.
AUTHOR: Pinol-Roma S; Choi Y D; Matunis M J; Dreyfuss G
CORPORATE SOURCE: Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, Illinois 60208.
CONTRACT NUMBER: GM-31888 (NIGMS)
GM-37125 (NIGMS)
SOURCE: Genes & development, (1988 Feb) Vol. 2, No. 2, pp. 215-27. Journal code: 8711660. ISSN: 0890-9369.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198806
ENTRY DATE: Entered STN: 8 Mar 1990
Last Updated on STN: 3 Feb 1997
Entered Medline: 2 Jun 1988

AB Heterogeneous nuclear RNA-ribonucleoprotein (hnRNP) particles can be efficiently purified by a specific, rapid, and mild procedure using monoclonal antibodies to hnRNP proteins. We report here on the detailed analysis of the protein composition of immunopurified hnRNP particles from human HeLa cells. By two-dimensional gel electrophoresis, immunopurified hnRNP particles contain at least 24 polypeptides in the range of 34,000-120,000 daltons. The abundant 30,000-40,000 dalton proteins, A, B, and C, described previously, are a subset of these polypeptides. The protein compositions of hnRNP particles found in the nucleoplasm fraction and in the chromatin-nucleolar fraction are very similar. Upon addition of the polyanion heparin, most of the major proteins remain associated in heparin-resistant particles, and only several, mostly minor, proteins dissociate. This provides an aid in the classification of the proteins and an additional criterion for the definition of hnRNP particle components. Chromatography on single-stranded DNA (ssDNA)-agarose in a heparin- and moderate or high salt (higher than 300 mM NaCl)-resistant manner suggests that most, if not all, of these proteins are single-stranded nucleic acid-binding proteins. We describe a general method for the large-scale purification of hnRNP proteins by affinity chromatography on ssDNA columns and its use for the production of new monoclonal antibodies to hnRNP proteins
.

L11 ANSWER 8 OF 8 MEDLINE on STN
ACCESSION NUMBER: 78084417 MEDLINE
DOCUMENT NUMBER: PubMed ID: 563790
TITLE: A new method for the purification of RNA polymerase II (or B) from the lower eukaryote Physarum polycephalum. The presence of subforms.
AUTHOR: Smith S S; Braun R
SOURCE: European journal of biochemistry / FEBS, (1978 Jan 2) Vol. 82, No. 1, pp. 309-20.
Journal code: 0107600. ISSN: 0014-2956.
PUB. COUNTRY: GERMANY, WEST: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 197803
ENTRY DATE: Entered STN: 14 Mar 1990
Last Updated on STN: 29 Jan 1999
Entered Medline: 29 Mar 1978

AB The DNA-dependent RNA polymerase II or B from the lower eukaryote Physarum polycephalum has been purified to apparent homogeneity by a new method employing poly(ethylene imine) precipitation and elution, and heparin-Sepharose affinity chromatography. The method is readily scaled up or down and affords a purification of over 5000-fold with a yield of 35-45%. The procedure is easy to perform and can be carried out in less than three days even on a large scale. Furthermore, it gives enzyme of higher purity and in at least 10-fold greater yield than previously published procedures for its purification from this organism. These improvements have allowed the detection of a series of subforms of the enzyme. The combination of precipitation using poly(ethylene imine) with chromatography on heparin-Sepharose may prove useful in the preparation of other proteins which interact with nucleic acids.

L14 ANSWER 1 OF 10 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2005:684045 CAPLUS
DOCUMENT NUMBER: 143:262124
TITLE: Presence of the nucleic acid channel in renal brush-border membranes: Allosteric modulation by extracellular calcium
AUTHOR(S): Leal-Pinto, Edgar; Teixeira, Avelino; Tran, Baohuong; Hanss, Basil; Klotman, Paul E.
CORPORATE SOURCE: Division of Nephrology, Department of Medicine, Mount Sinai School of Medicine, New York, NY, USA
SOURCE: American Journal of Physiology (2005), 289(1, Pt. 2), F97-F106
CODEN: AJPHAP; ISSN: 0002-9513
PUBLISHER: American Physiological Society
DOCUMENT TYPE: Journal
LANGUAGE: English
AB We have previously described a cell surface channel complex that is highly selective for nucleic acid (6, 7). The channel complex was purified to homogeneity by solubilizing renal brush-border membranes (BBM) with CHAPS and separation by liquid chromatog. It was characterized by reconstitution in planar lipid bilayers. The channel consists of a pore-forming subunit that is blocked by heparan sulfate and a regulatory subunit that is blocked by L-malate (7). The current studies were performed to compare the characteristics of the nucleic acid-conducting channel in native BBM with the characteristics that have been determined for the complex reconstituted from purified proteins. BBM were purified by differential centrifugation and reconstituted in lipid bilayers. Current was not observed until oligodeoxynucleotide (ODN) was added. Conductance was 9.1±0.9 pS; rectification and voltage dependence were not observed Reversal potential (Erev) shifted to +14±0.1 mV by a 10-fold gradient for ODN but was not altered when gradients were created for any other ion. Open probability increased significantly with an increase in Ca2+ on the trans chamber of the bilayer apparatus Changes in cis Ca2+ were without effect. Addition of L-malate to the cis chamber or heparan sulfate to the trans chamber significantly reduced the open probability of the channel. These data demonstrate that the nucleic acid channel in BBM is electrophysiol. and pharmacol. identical to that previously reported for purified protein and demonstrate that a nucleic acid-conducting channel is a component of renal BBM.
REFERENCE COUNT: 32 THERE ARE 32 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L14 ANSWER 2 OF 10 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2005:625328 CAPLUS
DOCUMENT NUMBER: 143:224523
TITLE: Protease-resistant Prion Protein Amplification Reconstituted with Partially Purified Substrates and Synthetic Polyanions
AUTHOR(S): Deleault, Nathan R.; Geoghegan, James C.; Nishina, Koren; Kascsak, Richard; Williamson, R. Anthony; Supattapone, Surachai
CORPORATE SOURCE: Department of Biochemistry, Dartmouth Medical School, Hanover, NH, 03755, USA
SOURCE: Journal of Biological Chemistry (2005), 280(29), 26873-26879
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Little is currently known about the biochem. mechanism by which induced

prion protein (PrP) conformational change occurs during mammalian prion propagation. In this study, we describe the reconstitution of PrPres amplification in vitro using partially purified and synthetic components. Overnight incubation of purified PrP27-30 and PrPC mols. at a molar ratio of 1:250 yielded .apprx.2-fold baseline PrPres amplification. Addition of various polyanionic mols. increased the level of PrPres amplification to .apprx.10-fold overall. Polyanionic compds. that stimulated purified PrPres amplification to varying degrees included synthetic, homopolymeric nucleic acids such as poly(A) and poly(dT), as well as non-nucleic acid polyanions, such as heparan sulfate proteoglycan. Size fractionation expts. showed that synthetic poly(A) polymers must be >0.2 kb in length to stimulate purified PrPres amplification. Thus, one possible set of minimal components for efficient conversion of PrP mols. in vitro may be surprisingly simple, consisting of PrP27-30, PrPC, and a stimulatory polyanionic compound

REFERENCE COUNT: 47 THERE ARE 47 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L14 ANSWER 3 OF 10 CAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 2004:41607 CAPLUS

DOCUMENT NUMBER: 140:107498

TITLE: Protein and cDNA sequences of a novel human heparin sulfate D-glucosaminyl-3-O-sulfotransferase isoform 5 (3-OST-5) and use in therapy and drug screening

INVENTOR(S): Xia, Guoqing; Malmstrom, Anders; Liu, Jian; Chen, Jinghua; Duncan, Michael B.; Shukla, Deepak; Tiwari, Vaibhav

PATENT ASSIGNEE(S): University of North Carolina at Chapel Hill, USA; The Board of Trustees of the University of Illinois

SOURCE: PCT Int. Appl., 143 pp.
CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | | DATE |
|---|---|---|---|---|---|
| WO 2004005475 | A2 | 20040115 | WO 2003-US21094 | | 20030707 |
| WO 2004005475 | A3 | 20041202 | | | |
| W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW | | | | | |
| RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG | | | | | |
| AU 2003247808 | A1 | 20040123 | AU 2003-247808 | | 20030707 |
| PRIORITY APPLN. INFO.: | | | US 2002-394199P | P | 20020705 |
| | | | WO 2003-US21094 | W | 20030707 |

AB The invention provides protein and cDNA sequences of a novel human heparin sulfate D-glucosaminyl-3-O-sulfotransferase isoform 5. Recombinant host cells, recombinant nucleic acids and recombinant proteins are also disclosed, along with methods of producing each. Isolated and purified antibodies to 3-OST-5 homologs, and methods of producing the same, are also disclosed. 3-OST-5 gene products have biol. activity in specific heparan sulfate 3-O-sulfotransferase reactions. These reactions provide unique modified heparan sulfate. Thus, therapeutic methods involving this activity are also disclosed.

L14 ANSWER 4 OF 10 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1998:122020 CAPLUS
DOCUMENT NUMBER: 128:279797
TITLE: Identification and characterization of a cell membrane nucleic acid channel
AUTHOR(S): Hanss, Basil; Leal-Pinto, Edgar; Bruggeman, Leslie A.; Copeland, Terry D.; Klotman, Paul E.
CORPORATE SOURCE: Division of Nephrology, Mt. Sinai School of Medicine, New York, NY, 10029, USA
SOURCE: Proceedings of the National Academy of Sciences of the United States of America (1998), 95(4), 1921-1926
CODEN: PNASA6; ISSN: 0027-8424
PUBLISHER: National Academy of Sciences
DOCUMENT TYPE: Journal
LANGUAGE: English
AB We have identified a 45-kDa protein purified from rat renal brush border membrane that binds short single-stranded nucleic acid sequences. This activity was purified, reconstituted in proteoliposomes, and then fused with model planar lipid bilayers. In voltage-clamp expts., the reconstituted 45-kDa protein functioned as a gated channel that allows the passage of nucleic acids. Channel activity was observed immediately after addition of oligonucleotide. Channel activity was not observed in the absence of purified protein or of oligonucleotide or when protein was heat-inactivated prior to forming proteoliposomes. In the presence of sym. buffered solution and oligonucleotide, current passed linearly over the range of holding potentials tested. Conductance was 10.4±0.4 picosiemens (pS) and reversal potential was 0.2±1.7 mV. There was no difference in channel conductance or reversal potential between phosphodiester and phosphorothioate oligonucleotides. Ion substitution expts. documented a shift in reversal potential only when a concentration gradient for oligonucleotide was established, indicating that movement of oligonucleotide alone was responsible for current. Movement of oligonucleotide across the bilayer was confirmed by using 32P-labeled oligonucleotides. Channel open probability decreased significantly in the presence of heparan sulfate. These studies provide evidence for a cell surface channel that conducts nucleic acids.
REFERENCE COUNT: 36 THERE ARE 36 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L14 ANSWER 5 OF 10 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1997:729656 CAPLUS
DOCUMENT NUMBER: 128:58940
TITLE: Molecular cloning and expression of mouse and human cDNAs encoding heparan sulfate D-glucosaminyl 3-O-sulfotransferase
AUTHOR(S): Shworak, Nicholas W.; Liu, Jian; Fritze, Linda M. S.; Schwartz, John J.; Zhang, Lijuan; Logeart, Delphine; Rosenberg, Robert D.
CORPORATE SOURCE: Department of Biology, Massachusetts Institute of Technology, Cambridge, MA, 02139, USA
SOURCE: Journal of Biological Chemistry (1997), 272(44), 28008-28019
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The cellular rate of anticoagulant heparan sulfate proteoglycan (HSPGact) generation is determined by the level of a kinetically limiting microsomal activity, HSact conversion activity, which is predominantly composed of the long sought heparan sulfate D-glucosaminyl

3-O-sulfotransferase (3-OST) (Shworak, N. W., Fritze, L. M. S., Liu, J., Butler, L. D., and Rosenberg, R. D. (1996) J. Biol. Chemical 271, 27063-27071; Liu, J., Shworak, N. W., Fritze, L. M. S., Edelberg, J. M., and Rosenberg, R. D. (1996) J. Biol. Chemical 271, 27072-27082). Mouse 3-OST cDNAs were isolated by proteolyzing the purified enzyme with Lys-C, sequencing the resultant peptides as well as the existing amino terminus, employing degenerate polymerase chain reaction primers corresponding to the sequences of the peptides as well as the amino terminus to amplify a fragment from LTA cDNA, and utilizing the resultant probe to obtain full-length enzyme cDNAs from a λ Zap Express LTA cDNA library. Human 3-OST cDNAs were isolated by searching the expressed sequence tag data bank with the mouse sequence, identifying a partial-length human cDNA and utilizing the clone as a probe to isolate a full-length enzyme cDNA from a λ TriplEx human brain cDNA library. The expression of wild-type mouse 3-OST as well as protein A-tagged mouse enzyme by transient transfection of COS-7 cells and the expression of both wild-type mouse and human 3-OST by in vitro transcription/translation demonstrate that the two cDNAs directly encode both HSact conversion and 3-OST activities. The mouse 3-OST cDNAs exhibit three different size classes because of a 5'-untranslated region of variable length, which results from the insertion of 0-1629 base pairs (bp) between residues 216 and 217; however, all cDNAs contain the same open reading frame of 933 bp. The length of the 3'-untranslated region ranges from 301 to 430 bp. The nucleic acid sequence of mouse and human 3-OST cDNAs are .apprx.85% similar, encoding novel 311- and 307-amino acid proteins of 35,876 and 35,750 Da, resp., that are 93% similar. The encoded enzymes are predicted to be intraluminal Golgi residents, presumably interacting via their C-terminal regions with an integral membrane protein. Both 3-OST species exhibit five potential N-glycosylation sites, which account for the apparent discrepancy between the mol. masses of the encoded enzyme (.apprx.34 kDa) and the previously purified enzyme (.apprx.46 kDa). The two 3-OST species also exhibit .apprx.50% similarity with all previously identified forms of the heparan biosynthetic enzyme N-deacetylase/N-sulfotransferase, which suggests that heparan biosynthetic enzymes share a common sulfotransferase domain.

REFERENCE COUNT: 68 THERE ARE 68 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L14 ANSWER 6 OF 10 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1987:107899 CAPLUS
DOCUMENT NUMBER: 106:107899
TITLE: Isolation of heparan sulfate and dermatan sulfate from arteries and cardiac muscles
PATENT ASSIGNEE(S): Mediolanum Farmaceutici S.r.l., Italy
SOURCE: Jpn. Kokai Tokkyo Koho, 8 pp.
CODEN: JKXXAF
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| JP 61218601 | A2 | 19860929 | JP 1986-53838 | 19860313 |
| JP 02038601 | B4 | 19900831 | | |
| EP 199033 | A2 | 19861029 | EP 1986-102963 | 19860306 |
| EP 199033 | A3 | 19880330 | | |
| EP 199033 | B1 | 19940105 | | |
| R: AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE | | | | |
| AT 99702 | E | 19940115 | AT 1986-102963 | 19860306 |
| US 4783447 | A | 19881108 | US 1986-838133 | 19860310 |
| DK 8601138 | A | 19860914 | DK 1986-1138 | 19860312 |
| FI 8601020 | A | 19860914 | FI 1986-1020 | 19860312 |

| | | | | |
|---|---|---|---|---|
| FI 82700 | B | 19901231 | | |
| FI 82700 | C | 19910410 | | |
| NO 8600940 | A | 19860915 | NO 1986-940 | 19860312 |
| NO 166187 | B | 19910304 | | |
| NO 166187 | C | 19910612 | | |
| ZA 8601855 | A | 19861029 | ZA 1986-1855 | 19860312 |
| ES 552898 | A1 | 19870516 | ES 1986-552898 | 19860312 |
| IN 163616 | A | 19881015 | IN 1986-CA184 | 19860312 |
| CA 1286286 | A1 | 19910716 | CA 1986-503943 | 19860312 |
| AU 8654727 | A1 | 19860918 | AU 1986-54727 | 19860313 |
| AU 582221 | B2 | 19890316 | | |

PRIORITY APPLN. INFO.: IT 1985-19885 A 19850313
EP 1986-102963 A 19860306

AB Heparan sulfate and dermatan sulfate for treatment of venous thrombosis are isolated in pure form from arteries and cardiac muscles. The method comprises (1) extraction of proteoglycans from the tissues using urea in the extraction solution, (2) the solution is filtered, and urea eliminated, (3) mucopolysaccharides are separated from proteins, (4) proteins precipitated and filtered out, (5) nucleic acids eliminated, (6) mucopolysaccharides precipitated, and (7) heparan sulfate and dermatan sulfate extracted and purified. From 1000 kg of arteries and cardiac muscles of mammals, 240 g heparan sulfate and 150 g dermatan sulfate were obtained.

L14 ANSWER 7 OF 10 MEDLINE on STN
ACCESSION NUMBER: 2005369851 MEDLINE
DOCUMENT NUMBER: PubMed ID: 15917229
TITLE: Protease-resistant prion protein amplification reconstituted with partially purified substrates and synthetic polyanions.
AUTHOR: Deleault Nathan R; Geoghegan James C; Nishina Koren; Kascsak Richard; Williamson R Anthony; Supattapone Surachai
CORPORATE SOURCE: Department of Biochemistry, Dartmouth Medical School, Hanover, New Hampshire 03755, USA.
CONTRACT NUMBER: AI058979 (NIAID)
NS046478 (NINDS)
SOURCE: The Journal of biological chemistry, (2005 Jul 22) Vol. 280, No. 29, pp. 26873-9. Electronic Publication: 2005-05-24.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200509
ENTRY DATE: Entered STN: 20 Jul 2005
Last Updated on STN: 11 Sep 2005
Entered Medline: 9 Sep 2005

AB Little is currently known about the biochemical mechanism by which induced prion protein (PrP) conformational change occurs during mammalian prion propagation. In this study, we describe the reconstitution of PrPres amplification in vitro using partially purified and synthetic components. Overnight incubation of purified PrP27-30 and PrPC molecules at a molar ratio of 1:250 yielded approximately 2-fold baseline PrPres amplification. Addition of various polyanionic molecules increased the level of PrPres amplification to approximately 10-fold overall. Polyanionic compounds that stimulated purified PrPres amplification to varying degrees included synthetic, homopolymeric nucleic acids such as poly(A) and poly(dT), as well as non-nucleic acid polyanions, such as heparan sulfate proteoglycan. Size fractionation experiments showed that synthetic poly(A) polymers must be >0.2 kb in length to stimulate purified PrPres amplification. Thus, one

possible set of minimal components for efficient conversion of PrP molecules in vitro may be surprisingly simple, consisting of PrP27-30, PrPC, and a stimulatory polyanionic compound.

L14 ANSWER 8 OF 10 MEDLINE on STN
ACCESSION NUMBER: 2005302368 MEDLINE
DOCUMENT NUMBER: PubMed ID: 15727991
TITLE: Presence of the nucleic acid channel in renal brush-border membranes: allosteric modulation by extracellular calcium.
AUTHOR: Leal-Pinto Edgar; Teixeira Avelino; Tran Baohuong; Hanss Basil; Klotman Paul E
CORPORATE SOURCE: Division of Nephrology, Mt. Sinai School of Medicine, New York, NY 10029, USA.
CONTRACT NUMBER: 1P0-1DK-50795 (NIDDK)
DK-63610-01 (NIDDK)
SOURCE: American journal of physiology. Renal physiology, (2005 Jul) Vol. 289, No. 1, pp. F97-106. Electronic Publication: 2005-02-22.
Journal code: 100901990. ISSN: 0363-6127.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200507
ENTRY DATE: Entered STN: 14 Jun 2005
Last Updated on STN: 12 Jul 2005
Entered Medline: 11 Jul 2005

AB We have previously described a cell surface channel complex that is highly selective for nucleic acid (6, 7). The channel complex was purified to homogeneity by solubilizing renal brush-border membranes (BBM) with CHAPS and separation by liquid chromatography. It was characterized by reconstitution in planar lipid bilayers. The channel consists of a pore-forming subunit that is blocked by heparan sulfate and a regulatory subunit that is blocked by L-malate (7). The current studies were performed to compare the characteristics of the nucleic acid-conducting channel in native BBM with the characteristics that have been determined for the complex reconstituted from purified proteins. BBM were purified by differential centrifugation and reconstituted in lipid bilayers. Current was not observed until oligodeoxynucleotide (ODN) was added. Conductance was 9.1 +/- 0.9 pS; rectification and voltage dependence were not observed. Reversal potential (E(rev)) shifted to +14 +/- 0.1 mV by a 10-fold gradient for ODN but was not altered when gradients were created for any other ion. Open probability increased significantly with an increase in Ca(2+) on the trans chamber of the bilayer apparatus. Changes in cis Ca(2+) were without effect. Addition of L-malate to the cis chamber or heparan sulfate to the trans chamber significantly reduced the open probability of the channel. These data demonstrate that the nucleic acid channel in BBM is electrophysiologically and pharmacologically identical to that previously reported for purified protein and demonstrate that a nucleic acid-conducting channel is a component of renal BBM.

L14 ANSWER 9 OF 10 MEDLINE on STN
ACCESSION NUMBER: 1998132693 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9465118
TITLE: Identification and characterization of a cell membrane nucleic acid channel.
AUTHOR: Hanss B; Leal-Pinto E; Bruggeman L A; Copeland T D; Klotman P E
CORPORATE SOURCE: Division of Nephrology, Box 1243, Mt. Sinai School of Medicine, 1 Gustave L. Levy Place, New York, NY 10029, USA.. b_hanss@smtplink.mssm.edu

CONTRACT NUMBER: 1PO1DK50795-02 (NIDDK)
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (1998 Feb 17) Vol. 95, No. 4, pp. 1921-6.
Journal code: 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199803
ENTRY DATE: Entered STN: 26 Mar 1998
Last Updated on STN: 26 Mar 1998
Entered Medline: 19 Mar 1998

AB We have identified a 45-kDa protein purified from rat renal brush border membrane that binds short single-stranded nucleic acid sequences. This activity was purified, reconstituted in proteoliposomes, and then fused with model planar lipid bilayers. In voltage-clamp experiments, the reconstituted 45-kDa protein functioned as a gated channel that allows the passage of nucleic acids. Channel activity was observed immediately after addition of oligonucleotide. Channel activity was not observed in the absence of purified protein or of oligonucleotide or when protein was heat-inactivated prior to forming proteoliposomes. In the presence of symmetrical buffered solution and oligonucleotide, current passed linearly over the range of holding potentials tested. Conductance was 10.4 +/- 0.4 picosiemens (pS) and reversal potential was 0.2 +/- 1.7 mV. There was no difference in channel conductance or reversal potential between phosphodiester and phosphorothioate oligonucleotides. Ion-substitution experiments documented a shift in reversal potential only when a concentration gradient for oligonucleotide was established, indicating that movement of oligonucleotide alone was responsible for current. Movement of oligonucleotide across the bilayer was confirmed by using 32P-labeled oligonucleotides. Channel open probability decreased significantly in the presence of heparan sulfate. These studies provide evidence for a cell surface channel that conducts nucleic acids.

L14 ANSWER 10 OF 10 MEDLINE on STN
ACCESSION NUMBER: 1998010647 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9346953
TITLE: Molecular cloning and expression of mouse and human cDNAs encoding heparan sulfate D-glucosaminyl 3-O-sulfotransferase.
AUTHOR: Shworak N W; Liu J; Fritze L M; Schwartz J J; Zhang L; Logeart D; Rosenberg R D
CORPORATE SOURCE: Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA.
CONTRACT NUMBER: HL-41484 (NHLBI)
SOURCE: The Journal of biological chemistry, (1997 Oct 31) Vol. 272, No. 44, pp. 28008-19.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF019385; GENBANK-AF019386
ENTRY MONTH: 199712
ENTRY DATE: Entered STN: 9 Jan 1998
Last Updated on STN: 9 Jan 1998
Entered Medline: 9 Dec 1997

AB The cellular rate of anticoagulant heparan sulfate proteoglycan (HSPGact) generation is determined by the level of a kinetically limiting microsomal activity, HSact conversion activity, which is predominantly

composed of the long sought heparan sulfate D-glucosaminyl 3-O-sulfotransferase (3-OST) (Shworak, N. W., Fritze, L. M. S., Liu, J., Butler, L. D., and Rosenberg, R. D. (1996) J. Biol. Chemical 271, 27063-27071; Liu, J., Shworak, N. W., Fritze, L. M. S., Edelberg, J. M., and Rosenberg, R. D. (1996) J. Biol. Chemical 271, 27072-27082). Mouse 3-OST cDNAs were isolated by proteolyzing the purified enzyme with Lys-C, sequencing the resultant peptides as well as the existing amino terminus, employing degenerate polymerase chain reaction primers corresponding to the sequences of the peptides as well as the amino terminus to amplify a fragment from LTA cDNA, and utilizing the resultant probe to obtain full-length enzyme cDNAs from a lambda Zap Express LTA cDNA library. Human 3-OST cDNAs were isolated by searching the expressed sequence tag data bank with the mouse sequence, identifying a partial-length human cDNA and utilizing the clone as a probe to isolate a full-length enzyme cDNA from a lambda TriplEx human brain cDNA library. The expression of wild-type mouse 3-OST as well as protein A-tagged mouse enzyme by transient transfection of COS-7 cells and the expression of both wild-type mouse and human 3-OST by in vitro transcription/translation demonstrate that the two cDNAs directly encode both HSact conversion and 3-OST activities. The mouse 3-OST cDNAs exhibit three different size classes because of a 5'-untranslated region of variable length, which results from the insertion of 0-1629 base pairs (bp) between residues 216 and 217; however, all cDNAs contain the same open reading frame of 933 bp. The length of the 3'-untranslated region ranges from 301 to 430 bp. The nucleic acid sequence of mouse and human 3-OST cDNAs are approximately 85% similar, encoding novel 311- and 307-amino acid proteins of 35,876 and 35,750 daltons, respectively, that are 93% similar. The encoded enzymes are predicted to be intraluminal Golgi residents, presumably interacting via their C-terminal regions with an integral membrane protein. Both 3-OST species exhibit five potential N-glycosylation sites, which account for the apparent discrepancy between the molecular masses of the encoded enzyme (approximately 34 kDa) and the previously purified enzyme (approximately 46 kDa). The two 3-OST species also exhibit approximately 50% similarity with all previously identified forms of the heparan biosynthetic enzyme N-deacetylase/N-sulfotransferase, which suggests that heparan biosynthetic enzymes share a common sulfotransferase domain.

L14 ANSWER 1 OF 10 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2005:684045 CAPLUS
DOCUMENT NUMBER: 143:262124
TITLE: Presence of the nucleic acid channel in renal brush-border membranes: Allosteric modulation by extracellular calcium
AUTHOR(S): Leal-Pinto, Edgar; Teixeira, Avelino; Tran, Baohuong; Hanss, Basil; Klotman, Paul E.
CORPORATE SOURCE: Division of Nephrology, Department of Medicine, Mount Sinai School of Medicine, New York, NY, USA
SOURCE: American Journal of Physiology (2005), 289(1, Pt. 2), F97-F106
CODEN: AJPHAP; ISSN: 0002-9513
PUBLISHER: American Physiological Society
DOCUMENT TYPE: Journal
LANGUAGE: English
AB We have previously described a cell surface channel complex that is highly selective for nucleic acid (6, 7). The channel complex was purified to homogeneity by solubilizing renal brush-border membranes (BBM) with CHAPS and separation by liquid chromatog. It was characterized by reconstitution in planar lipid bilayers. The channel consists of a pore-forming subunit that is blocked by heparan sulfate and a regulatory subunit that is blocked by L-malate (7). The current studies were performed to compare the characteristics of the nucleic acid-conducting channel in native BBM with the characteristics that have been determined for the complex reconstituted from purified proteins. BBM were purified by differential centrifugation and reconstituted in lipid bilayers. Current was not observed until oligodeoxynucleotide (ODN) was added. Conductance was 9.1±0.9 pS; rectification and voltage dependence were not observed Reversal potential (Erev) shifted to +14±0.1 mV by a 10-fold gradient for ODN but was not altered when gradients were created for any other ion. Open probability increased significantly with an increase in Ca2+ on the trans chamber of the bilayer apparatus Changes in cis Ca2+ were without effect. Addition of L-malate to the cis chamber or heparan sulfate to the trans chamber significantly reduced the open probability of the channel. These data demonstrate that the nucleic acid channel in BBM is electrophysiol. and pharmacol. identical to that previously reported for purified protein and demonstrate that a nucleic acid-conducting channel is a component of renal BBM.
REFERENCE COUNT: 32 THERE ARE 32 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L14 ANSWER 2 OF 10 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2005:625328 CAPLUS
DOCUMENT NUMBER: 143:224523
TITLE: Protease-resistant Prion Protein Amplification Reconstituted with Partially Purified Substrates and Synthetic Polyanions
AUTHOR(S): Deleault, Nathan R.; Geoghegan, James C.; Nishina, Koren; Kascsak, Richard; Williamson, R. Anthony; Supattapone, Surachai
CORPORATE SOURCE: Department of Biochemistry, Dartmouth Medical School, Hanover, NH, 03755, USA
SOURCE: Journal of Biological Chemistry (2005), 280(29), 26873-26879
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Little is currently known about the biochem. mechanism by which induced

prion protein (PrP) conformational change occurs during mammalian prion propagation. In this study, we describe the reconstitution of PrPres amplification in vitro using partially purified and synthetic components. Overnight incubation of purified PrP27-30 and PrPC mols. at a molar ratio of 1:250 yielded .apprx.2-fold baseline PrPres amplification. Addition of various polyanionic mols. increased the level of PrPres amplification to .apprx.10-fold overall. Polyanionic compds. that stimulated purified PrPres amplification to varying degrees included synthetic, homopolymeric nucleic acids such as poly(A) and poly(dT), as well as non-nucleic acid polyanions, such as heparan sulfate proteoglycan. Size fractionation expts. showed that synthetic poly(A) polymers must be >0.2 kb in length to stimulate purified PrPres amplification. Thus, one possible set of minimal components for efficient conversion of PrP mols. in vitro may be surprisingly simple, consisting of PrP27-30, PrPC, and a stimulatory polyanionic compound

REFERENCE COUNT: 47 THERE ARE 47 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L14 ANSWER 3 OF 10 CAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 2004:41607 CAPLUS

DOCUMENT NUMBER: 140:107498

TITLE: Protein and cDNA sequences of a novel human heparin sulfate D-glucosaminyl-3-O-sulfotransferase isoform 5 (3-OST-5) and use in therapy and drug screening

INVENTOR(S): Xia, Guoqing; Malmstrom, Anders; Liu, Jian; Chen, Jinghua; Duncan, Michael B.; Shukla, Deepak; Tiwari, Vaibhav

PATENT ASSIGNEE(S): University of North Carolina at Chapel Hill, USA; The Board of Trustees of the University of Illinois

SOURCE: PCT Int. Appl., 143 pp.
CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | | DATE |
|---|---|---|---|---|---|
| WO 2004005475 | A2 | 20040115 | WO 2003-US21094 | | 20030707 |
| WO 2004005475 | A3 | 20041202 | | | |
| W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW | | | | | |
| RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG | | | | | |
| AU 2003247808 | A1 | 20040123 | AU 2003-247808 | | 20030707 |
| PRIORITY APPLN. INFO.: | | | US 2002-394199P | P | 20020705 |
| | | | WO 2003-US21094 | W | 20030707 |

AB The invention provides protein and cDNA sequences of a novel human heparin sulfate D-glucosaminyl-3-O-sulfotransferase isoform 5. Recombinant host cells, recombinant nucleic acids and recombinant proteins are also disclosed, along with methods of producing each. Isolated and purified antibodies to 3-OST-5 homologs, and methods of producing the same, are also disclosed. 3-OST-5 gene products have biol. activity in specific heparan sulfate 3-O-sulfotransferase reactions. These reactions provide unique modified heparan sulfate. Thus, therapeutic methods involving this activity are also disclosed.

L14 ANSWER 4 OF 10 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1998:122020 CAPLUS
DOCUMENT NUMBER: 128:279797
TITLE: Identification and characterization of a cell membrane nucleic acid channel
AUTHOR(S): Hanss, Basil; Leal-Pinto, Edgar; Bruggeman, Leslie A.; Copeland, Terry D.; Klotman, Paul E.
CORPORATE SOURCE: Division of Nephrology, Mt. Sinai School of Medicine, New York, NY, 10029, USA
SOURCE: Proceedings of the National Academy of Sciences of the United States of America (1998), 95(4), 1921-1926
CODEN: PNASA6; ISSN: 0027-8424
PUBLISHER: National Academy of Sciences
DOCUMENT TYPE: Journal
LANGUAGE: English
AB We have identified a 45-kDa protein purified from rat renal brush border membrane that binds short single-stranded nucleic acid sequences. This activity was purified, reconstituted in proteoliposomes, and then fused with model planar lipid bilayers. In voltage-clamp expts., the reconstituted 45-kDa protein functioned as a gated channel that allows the passage of nucleic acids. Channel activity was observed immediately after addition of oligonucleotide. Channel activity was not observed in the absence of purified protein or of oligonucleotide or when protein was heat-inactivated prior to forming proteoliposomes. In the presence of sym. buffered solution and oligonucleotide, current passed linearly over the range of holding potentials tested. Conductance was 10.4±0.4 picosiemens (pS) and reversal potential was 0.2±1.7 mV. There was no difference in channel conductance or reversal potential between phosphodiester and phosphorothioate oligonucleotides. Ion substitution expts. documented a shift in reversal potential only when a concentration gradient for oligonucleotide was established, indicating that movement of oligonucleotide alone was responsible for current. Movement of oligonucleotide across the bilayer was confirmed by using 32P-labeled oligonucleotides. Channel open probability decreased significantly in the presence of heparan sulfate. These studies provide evidence for a cell surface channel that conducts nucleic acids.
REFERENCE COUNT: 36 THERE ARE 36 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L14 ANSWER 5 OF 10 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1997:729656 CAPLUS
DOCUMENT NUMBER: 128:58940
TITLE: Molecular cloning and expression of mouse and human cDNAs encoding heparan sulfate D-glucosaminyl 3-O-sulfotransferase
AUTHOR(S): Shworak, Nicholas W.; Liu, Jian; Fritze, Linda M. S.; Schwartz, John J.; Zhang, Lijuan; Logeart, Delphine; Rosenberg, Robert D.
CORPORATE SOURCE: Department of Biology, Massachusetts Institute of Technology, Cambridge, MA, 02139, USA
SOURCE: Journal of Biological Chemistry (1997), 272(44), 28008-28019
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The cellular rate of anticoagulant heparan sulfate proteoglycan (HSPGact) generation is determined by the level of a kinetically limiting microsomal activity, HSact conversion activity, which is predominantly composed of the long sought heparan sulfate D-glucosaminyl

3-O-sulfotransferase (3-OST) (Shworak, N. W., Fritze, L. M. S., Liu, J., Butler, L. D., and Rosenberg, R. D. (1996) J. Biol. Chemical 271, 27063-27071; Liu, J., Shworak, N. W., Fritze, L. M. S., Edelberg, J. M., and Rosenberg, R. D. (1996) J. Biol. Chemical 271, 27072-27082). Mouse 3-OST cDNAs were isolated by proteolyzing the purified enzyme with Lys-C, sequencing the resultant peptides as well as the existing amino terminus, employing degenerate polymerase chain reaction primers corresponding to the sequences of the peptides as well as the amino terminus to amplify a fragment from LTA cDNA, and utilizing the resultant probe to obtain full-length enzyme cDNAs from a λ Zap Express LTA cDNA library. Human 3-OST cDNAs were isolated by searching the expressed sequence tag data bank with the mouse sequence, identifying a partial-length human cDNA and utilizing the clone as a probe to isolate a full-length enzyme cDNA from a λ TriplEx human brain cDNA library. The expression of wild-type mouse 3-OST as well as protein A-tagged mouse enzyme by transient transfection of COS-7 cells and the expression of both wild-type mouse and human 3-OST by in vitro transcription/translation demonstrate that the two cDNAs directly encode both HSact conversion and 3-OST activities. The mouse 3-OST cDNAs exhibit three different size classes because of a 5'-untranslated region of variable length, which results from the insertion of 0-1629 base pairs (bp) between residues 216 and 217; however, all cDNAs contain the same open reading frame of 933 bp. The length of the 3'-untranslated region ranges from 301 to 430 bp. The nucleic acid sequence of mouse and human 3-OST cDNAs are .apprx.85% similar, encoding novel 311- and 307-amino acid proteins of 35,876 and 35,750 Da, resp., that are 93% similar. The encoded enzymes are predicted to be intraluminal Golgi residents, presumably interacting via their C-terminal regions with an integral membrane protein. Both 3-OST species exhibit five potential N-glycosylation sites, which account for the apparent discrepancy between the mol. masses of the encoded enzyme (.apprx.34 kDa) and the previously purified enzyme (.apprx.46 kDa). The two 3-OST species also exhibit .apprx.50% similarity with all previously identified forms of the heparan biosynthetic enzyme N-deacetylase/N-sulfotransferase, which suggests that heparan biosynthetic enzymes share a common sulfotransferase domain.

REFERENCE COUNT: 68 THERE ARE 68 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L14 ANSWER 6 OF 10 CAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 1987:107899 CAPLUS
DOCUMENT NUMBER: 106:107899
TITLE: Isolation of heparan sulfate and dermatan sulfate from arteries and cardiac muscles
PATENT ASSIGNEE(S): Mediolanum Farmaceutici S.r.l., Italy
SOURCE: Jpn. Kokai Tokkyo Koho, 8 pp.
CODEN: JKXXAF
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| JP 61218601 | A2 | 19860929 | JP 1986-53838 | 19860313 |
| JP 02038601 | B4 | 19900831 | | |
| EP 199033 | A2 | 19861029 | EP 1986-102963 | 19860306 |
| EP 199033 | A3 | 19880330 | | |
| EP 199033 | B1 | 19940105 | | |
| R: AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE | | | | |
| AT 99702 | E | 19940115 | AT 1986-102963 | 19860306 |
| US 4783447 | A | 19881108 | US 1986-838133 | 19860310 |
| DK 8601138 | A | 19860914 | DK 1986-1138 | 19860312 |
| FI 8601020 | A | 19860914 | FI 1986-1020 | 19860312 |

| | | | | |
|---|---|---|---|---|
| FI 82700 | B | 19901231 | | |
| FI 82700 | C | 19910410 | | |
| NO 8600940 | A | 19860915 | NO 1986-940 | 19860312 |
| NO 166187 | B | 19910304 | | |
| NO 166187 | C | 19910612 | | |
| ZA 8601855 | A | 19861029 | ZA 1986-1855 | 19860312 |
| ES 552898 | A1 | 19870516 | ES 1986-552898 | 19860312 |
| IN 163616 | A | 19881015 | IN 1986-CA184 | 19860312 |
| CA 1286286 | A1 | 19910716 | CA 1986-503943 | 19860312 |
| AU 8654727 | A1 | 19860918 | AU 1986-54727 | 19860313 |
| AU 582221 | B2 | 19890316 | | |

PRIORITY APPLN. INFO.: IT 1985-19885 A 19850313
EP 1986-102963 A 19860306

AB Heparan sulfate and dermatan sulfate for treatment of venous thrombosis are isolated in pure form from arteries and cardiac muscles. The method comprises (1) extraction of proteoglycans from the tissues using urea in the extraction solution, (2) the solution is filtered, and urea eliminated, (3) mucopolysaccharides are separated from proteins, (4) proteins precipitated and filtered out, (5) nucleic acids eliminated, (6) mucopolysaccharides precipitated, and (7) heparan sulfate and dermatan sulfate extracted and purified. From 1000 kg of arteries and cardiac muscles of mammals, 240 g heparan sulfate and 150 g dermatan sulfate were obtained.

L14 ANSWER 7 OF 10 MEDLINE on STN
ACCESSION NUMBER: 2005369851 MEDLINE
DOCUMENT NUMBER: PubMed ID: 15917229
TITLE: Protease-resistant prion protein amplification reconstituted with partially purified substrates and synthetic polyanions.
AUTHOR: Deleault Nathan R; Geoghegan James C; Nishina Koren; Kascsak Richard; Williamson R Anthony; Supattapone Surachai
CORPORATE SOURCE: Department of Biochemistry, Dartmouth Medical School, Hanover, New Hampshire 03755, USA.
CONTRACT NUMBER: AI058979 (NIAID)
NS046478 (NINDS)
SOURCE: The Journal of biological chemistry, (2005 Jul 22) Vol. 280, No. 29, pp. 26873-9. Electronic Publication: 2005-05-24.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200509
ENTRY DATE: Entered STN: 20 Jul 2005
Last Updated on STN: 11 Sep 2005
Entered Medline: 9 Sep 2005

AB Little is currently known about the biochemical mechanism by which induced prion protein (PrP) conformational change occurs during mammalian prion propagation. In this study, we describe the reconstitution of PrPres amplification in vitro using partially purified and synthetic components. Overnight incubation of purified PrP27-30 and PrPC molecules at a molar ratio of 1:250 yielded approximately 2-fold baseline PrPres amplification. Addition of various polyanionic molecules increased the level of PrPres amplification to approximately 10-fold overall. Polyanionic compounds that stimulated purified PrPres amplification to varying degrees included synthetic, homopolymeric nucleic acids such as poly(A) and poly(dT), as well as non-nucleic acid polyanions, such as heparan sulfate proteoglycan. Size fractionation experiments showed that synthetic poly(A) polymers must be >0.2 kb in length to stimulate purified PrPres amplification. Thus, one

possible set of minimal components for efficient conversion of PrP molecules in vitro may be surprisingly simple, consisting of PrP27-30, PrPC, and a stimulatory polyanionic compound.

L14 ANSWER 8 OF 10 MEDLINE on STN
ACCESSION NUMBER: 2005302368 MEDLINE
DOCUMENT NUMBER: PubMed ID: 15727991
TITLE: Presence of the nucleic acid channel in renal brush-border membranes: allosteric modulation by extracellular calcium.
AUTHOR: Leal-Pinto Edgar; Teixeira Avelino; Tran Baohuong; Hanss Basil; Klotman Paul E
CORPORATE SOURCE: Division of Nephrology, Mt. Sinai School of Medicine, New York, NY 10029, USA.
CONTRACT NUMBER: 1P0-1DK-50795 (NIDDK)
DK-63610-01 (NIDDK)
SOURCE: American journal of physiology. Renal physiology, (2005 Jul) Vol. 289, No. 1, pp. F97-106. Electronic Publication: 2005-02-22.
Journal code: 100901990. ISSN: 0363-6127.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200507
ENTRY DATE: Entered STN: 14 Jun 2005
Last Updated on STN: 12 Jul 2005
Entered Medline: 11 Jul 2005

AB We have previously described a cell surface channel complex that is highly selective for nucleic acid (6, 7). The channel complex was purified to homogeneity by solubilizing renal brush-border membranes (BBM) with CHAPS and separation by liquid chromatography. It was characterized by reconstitution in planar lipid bilayers. The channel consists of a pore-forming subunit that is blocked by heparan sulfate and a regulatory subunit that is blocked by L-malate (7). The current studies were performed to compare the characteristics of the nucleic acid-conducting channel in native BBM with the characteristics that have been determined for the complex reconstituted from purified proteins. BBM were purified by differential centrifugation and reconstituted in lipid bilayers. Current was not observed until oligodeoxynucleotide (ODN) was added. Conductance was 9.1 +/- 0.9 pS; rectification and voltage dependence were not observed. Reversal potential (E(rev)) shifted to +14 +/- 0.1 mV by a 10-fold gradient for ODN but was not altered when gradients were created for any other ion. Open probability increased significantly with an increase in Ca(2+) on the trans chamber of the bilayer apparatus. Changes in cis Ca(2+) were without effect. Addition of L-malate to the cis chamber or heparan sulfate to the trans chamber significantly reduced the open probability of the channel. These data demonstrate that the nucleic acid channel in BBM is electrophysiologically and pharmacologically identical to that previously reported for purified protein and demonstrate that a nucleic acid-conducting channel is a component of renal BBM.

L14 ANSWER 9 OF 10 MEDLINE on STN
ACCESSION NUMBER: 1998132693 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9465118
TITLE: Identification and characterization of a cell membrane nucleic acid channel.
AUTHOR: Hanss B; Leal-Pinto E; Bruggeman L A; Copeland T D; Klotman P E
CORPORATE SOURCE: Division of Nephrology, Box 1243, Mt. Sinai School of Medicine, 1 Gustave L. Levy Place, New York, NY 10029, USA.. b_hanss@smtplink.mssm.edu

CONTRACT NUMBER: 1PO1DK50795-02 (NIDDK)
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (1998 Feb 17) Vol. 95, No. 4, pp. 1921-6.
Journal code: 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199803
ENTRY DATE: Entered STN: 26 Mar 1998
Last Updated on STN: 26 Mar 1998
Entered Medline: 19 Mar 1998

AB We have identified a 45-kDa protein purified from rat renal brush border membrane that binds short single-stranded nucleic acid sequences. This activity was purified, reconstituted in proteoliposomes, and then fused with model planar lipid bilayers. In voltage-clamp experiments, the reconstituted 45-kDa protein functioned as a gated channel that allows the passage of nucleic acids. Channel activity was observed immediately after addition of oligonucleotide. Channel activity was not observed in the absence of purified protein or of oligonucleotide or when protein was heat-inactivated prior to forming proteoliposomes. In the presence of symmetrical buffered solution and oligonucleotide, current passed linearly over the range of holding potentials tested. Conductance was 10.4 +/- 0.4 picosiemens (pS) and reversal potential was 0.2 +/- 1.7 mV. There was no difference in channel conductance or reversal potential between phosphodiester and phosphorothioate oligonucleotides. Ion-substitution experiments documented a shift in reversal potential only when a concentration gradient for oligonucleotide was established, indicating that movement of oligonucleotide alone was responsible for current. Movement of oligonucleotide across the bilayer was confirmed by using 32P-labeled oligonucleotides. Channel open probability decreased significantly in the presence of heparan sulfate. These studies provide evidence for a cell surface channel that conducts nucleic acids.

L14 ANSWER 10 OF 10 MEDLINE on STN
ACCESSION NUMBER: 1998010647 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9346953
TITLE: Molecular cloning and expression of mouse and human cDNAs encoding heparan sulfate D-glucosaminyl 3-O-sulfotransferase.
AUTHOR: Shworak N W; Liu J; Fritze L M; Schwartz J J; Zhang L; Logeart D; Rosenberg R D
CORPORATE SOURCE: Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA.
CONTRACT NUMBER: HL-41484 (NHLBI)
SOURCE: The Journal of biological chemistry, (1997 Oct 31) Vol. 272, No. 44, pp. 28008-19.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF019385; GENBANK-AF019386
ENTRY MONTH: 199712
ENTRY DATE: Entered STN: 9 Jan 1998
Last Updated on STN: 9 Jan 1998
Entered Medline: 9 Dec 1997

AB The cellular rate of anticoagulant heparan sulfate proteoglycan (HSPGact) generation is determined by the level of a kinetically limiting microsomal activity, HSact conversion activity, which is predominantly

composed of the long sought heparan sulfate D-glucosaminyl 3-O-sulfotransferase (3-OST) (Shworak, N. W., Fritze, L. M. S., Liu, J., Butler, L. D., and Rosenberg, R. D. (1996) J. Biol. Chemical 271, 27063-27071; Liu, J., Shworak, N. W., Fritze, L. M. S., Edelberg, J. M., and Rosenberg, R. D. (1996) J. Biol. Chemical 271, 27072-27082). Mouse 3-OST cDNAs were isolated by proteolyzing the purified enzyme with Lys-C, sequencing the resultant peptides as well as the existing amino terminus, employing degenerate polymerase chain reaction primers corresponding to the sequences of the peptides as well as the amino terminus to amplify a fragment from LTA cDNA, and utilizing the resultant probe to obtain full-length enzyme cDNAs from a lambda Zap Express LTA cDNA library. Human 3-OST cDNAs were isolated by searching the expressed sequence tag data bank with the mouse sequence, identifying a partial-length human cDNA and utilizing the clone as a probe to isolate a full-length enzyme cDNA from a lambda TriplEx human brain cDNA library. The expression of wild-type mouse 3-OST as well as protein A-tagged mouse enzyme by transient transfection of COS-7 cells and the expression of both wild-type mouse and human 3-OST by in vitro transcription/translation demonstrate that the two cDNAs directly encode both HSact conversion and 3-OST activities. The mouse 3-OST cDNAs exhibit three different size classes because of a 5'-untranslated region of variable length, which results from the insertion of 0-1629 base pairs (bp) between residues 216 and 217; however, all cDNAs contain the same open reading frame of 933 bp. The length of the 3'-untranslated region ranges from 301 to 430 bp. The nucleic acid sequence of mouse and human 3-OST cDNAs are approximately 85% similar, encoding novel 311- and 307-amino acid proteins of 35,876 and 35,750 daltons, respectively, that are 93% similar. The encoded enzymes are predicted to be intraluminal Golgi residents, presumably interacting via their C-terminal regions with an integral membrane protein. Both 3-OST species exhibit five potential N-glycosylation sites, which account for the apparent discrepancy between the molecular masses of the encoded enzyme (approximately 34 kDa) and the previously purified enzyme (approximately 46 kDa). The two 3-OST species also exhibit approximately 50% similarity with all previously identified forms of the heparan biosynthetic enzyme N-deacetylase/N-sulfotransferase, which suggests that heparan biosynthetic enzymes share a common sulfotransferase domain.

L15 ANSWER 1 OF 5 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1992:125378 CAPLUS
DOCUMENT NUMBER: 116:125378
TITLE: High molecular weight mucin-like glycoproteins of the bovine interphotoreceptor matrix
AUTHOR(S): Plantner, James J.
CORPORATE SOURCE: Sch. Med., Case West. Reserve Univ., Cleveland, OH, 44106, USA
SOURCE: Experimental Eye Research (1992), 54(1), 113-25
CODEN: EXERA6; ISSN: 0014-4835
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A very high-mol. weight mucin-like glycoprotein was isolated by gel filtration of interphotoreceptor matrix (IPM) from fresh bovine eyes and purified to apparent homogeneity by CsCl/guanidine hydrochloride (GuHCl) equilibrium d. gradient centrifugation. Although a mol. weight in excess of 107 Da is suggested by gel filtration, the presence of SDS or GuHCl did not alter its elution position, indicating that the large size was not simply due to aggregation. Treatment of this material with disulfide reagents, however, led to a decrease in mol. size. On a relative basis, substantially more of this glycoprotein is present in IPM prepared from retina than from retinal pigment epithelium. While the carbohydrate and amino acid composition are not those of a true mucin, the large size and many other properties are quite mucin-like. The carbohydrate composition suggests the presence of both N- and O-glycosidically linked sugar chains. The presence of a mucin-type O-glycosidic linkage is indicated by its suceptibility to alkaline cleavage, with concomitant loss of serine and threonine and increase in 240 nm absorbance; production of a fluorescent product on reaction with cyanoacetamide; lectin-binding properties; and production of N-acetylgalactosaminitol on alkaline borohydride elimination. This glycoprotein was digested by pronase and trypsin, confirming its protein nature, but was resistant to digestion with chondroitin ABC lyase, hyaluronidase and heparinase, as well as RNAase, indicating that these components were not present to any appreciable extent. ELISA for cartilage keratan sulfate was also neg. Centrifugation in CsCl/GuHCl gradients indicated a d. much lower than that of a proteoglycan or nucleic acid as well. In vitro biosynthetic studies suggest that both retina and retinal pigment epithelium may be major sources of material in the IPM. The elution patterns of radioactivity were strikingly similar to the UV elution patterns of IPM. The medium from retinal incubations contained very high mol. weight material which was resistant to enzymes which hydrolyze glycosaminoglycans, suggesting that retina may be the source of this high-mol. weight, mucin-like glycoprotein.

L15 ANSWER 2 OF 5 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1988:91226 CAPLUS
DOCUMENT NUMBER: 108:91226
TITLE: Densitometric assay of nanogram quantities of proteoglycans precipitated on nitrocellulose membrane with safranin O
AUTHOR(S): Lammi, Mikko; Tammi, Markku
CORPORATE SOURCE: Dep. Anat., Univ. Kuopio, Kuopio, SF-70211, Finland
SOURCE: Analytical Biochemistry (1988), 168(2), 352-7
CODEN: ANBCA2; ISSN: 0003-2697
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Proteoglycan (PG) and glycosaminoglycan (GAG) samples corresponding to a min. of 10 ng uronic acid were reliably quantified as ppts. with the cationic dye Safranin O, collected by vacuum-aided filtration onto a cellulose acetate/nitrate membrane in a standard 96-well dot assay apparatus The

reflectances of the precipitation dots were measured by automatic densitometric scanning of the membrane sheets. Standard GAGs produced reflectance values which were related to the number of anionic groups per unit disaccharide; hyaluronate and keratan sulfate gave lower values while heparin yielded values higher than those of chondroitin sulfates. The presence of 8M urea, 1% Triton X 100, 30% sucrose, 0.02% NaN3, or mixts. of proteinase inhibitors and various buffers did not markedly influence the reflectances, while 4M guanidinium chloride and 3M CsCl reduced the sensitivity of the assay to 30-50 ng. Samples containing SDS were not applicable because SDS precipitated with Safranin O. Proteins showed virtually no response, while nucleic acids gave significant although smaller reflectances than GAGs. Owing to its marked sensitivity and convenience, the method is particularly suitable for the detection of PGs during their preparative purifn. and fractionation as well as in various anal. assays.

L15 ANSWER 3 OF 5 CAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1969:487745 CAPLUS
DOCUMENT NUMBER: 71:87745
TITLE: Protein-polysaccharides from pig laryngeal cartilage. Extraction and purification
AUTHOR(S): Tsiganos, Constantine P.; Muir, Helen
CORPORATE SOURCE: Kennedy Inst. Rheumatol., London, UK
SOURCE: Biochemical Journal (1969), 113(5), 879-84
CODEN: BIJOAK; ISSN: 0264-6021
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Protein-polysaccharides of chondroitin sulfate were extracted from fresh laryngeal cartilage at pH 6.8 by 2 procedures. Procedure 1 consisted of brief low-speed homogenization in 0.15M (iso-osmotic) NaOAc and procedure 2 consisted of longer homogenization followed by prolonged extraction in 10% CaCl2 solution The protein-polysaccharides in both exts. were isolated and purified by precipitation with 9-aminoacridine-HCl. They were free from serum proteins, collagen, and nucleic acids and also of degradative enzymes. The absence of such enzymes was shown by viscosity measurements on solns. of protein-polysaccharides incubated for up to 24 hrs. at pH 4 and 6.8. Mannose, glucose, or fucose was not detected by paper chromatog. and only traces of sialic acid were present. The yield with procedure 2 was twice that with procedure 1 and the products differed in their protein and glucosamine contents. Hyaluronic acid was unlikely to have been precipitated at an acid pH, so the glucosamine was attributed to keratan sulfate, as serum proteins were absent. There was no free keratan sulfate in the preparation Both prepns. were heterogeneous in the ultracentrifuge, showing at least 3 components.

L15 ANSWER 4 OF 5 MEDLINE on STN
ACCESSION NUMBER: 92175078 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1541329
TITLE: High molecular weight mucin-like glycoproteins of the bovine interphotoreceptor matrix.
AUTHOR: Plantner J J
CORPORATE SOURCE: Lorand V. Johnson Laboratory for Research in Ophthalmology, Department of Surgery, Case Western Reserve University, Cleveland, OH 44106.
CONTRACT NUMBER: EY 06571 (NEI)
SOURCE: Experimental eye research, (1992 Jan) Vol. 54, No. 1, pp. 113-25.
Journal code: 0370707. ISSN: 0014-4835.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals

ENTRY MONTH: 199204
ENTRY DATE: Entered STN: 24 Apr 1992
Last Updated on STN: 24 Apr 1992
Entered Medline: 8 Apr 1992

AB A very high molecular weight mucin-like glycoprotein was isolated by gel filtration of interphotoreceptor matrix (IPM) from fresh bovine eyes and purified to apparent homogeneity by cesium chloride/guanidine hydrochloride (GuHCl) equilibrium density gradient centrifugation. Although a molecular weight in excess of 10(7) Da is suggested by gel filtration, the presence of SDS or GuHCl did not alter its elution position, indicating that the large size was not simply due to aggregation. Treatment of this material with disulfide reagents, however, led to a decrease in molecular size. On a relative basis, substantially more of this glycoprotein is present in IPM prepared from retina than from retinal pigment epithelium. While the carbohydrate and amino acid composition are not those of a true 'mucin', the large size and many other properties are quite 'mucin-like'. The carbohydrate composition suggests the presence of both N- and O-glycosidically linked sugar chains. The presence of a mucin-type O-glycosidic linkage is indicated by its susceptibility to alkaline cleavage, with concomitant loss of serine and threonine and increase in 240 nm absorbance; production of a fluorescent product upon reaction with cyanoacetamide; lectin binding properties; and production of N-acetylgalactosaminitol upon alkaline borohydride elimination. This glycoprotein was digested by pronase and trypsin, confirming its protein nature, but was resistant to digestion with chondroitin ABC lyase, hyaluronidase and heparinase, as well as RNAase, indicating that these components were not present to any appreciable extent. ELISA for cartilage keratan sulfate was also negative. Centrifugation in CsCl/GuHCl gradients indicated a density much lower than that of a proteoglycan or nucleic acid as well. In vitro biosynthetic studies suggest that both retina and retinal pigment epithelium may be major sources of material in the IPM. The elution patterns of radioactivity were strikingly similar to the UV elution patterns of IPM. The medium from retinal incubations contained very high molecular weight material which was resistant to enzymes which hydrolyse glycosaminoglycans, suggesting that retina may be the source of this high molecular weight, mucin-like glycoprotein.

L15 ANSWER 5 OF 5 MEDLINE on STN
ACCESSION NUMBER: 88207935 MEDLINE
DOCUMENT NUMBER: PubMed ID: 3129962
TITLE: Densitometric assay of nanogram quantities of proteoglycans precipitated on nitrocellulose membrane with Safranin O.
AUTHOR: Lammi M; Tammi M
CORPORATE SOURCE: Department of Anatomy, University of Kuopio, Finland.
SOURCE: Analytical biochemistry, (1988 Feb 1) Vol. 168, No. 2, pp. 352-7.
Journal code: 0370535. ISSN: 0003-2697.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198805
ENTRY DATE: Entered STN: 8 Mar 1990
Last Updated on STN: 8 Mar 1990
Entered Medline: 31 May 1988

AB Proteoglycan (PG) and glycosaminoglycan (GAG) samples corresponding to a minimum of 10 ng of uronic acid were reliably quantified as precipitates with the cationic dye Safranin O, collected by vacuum-aided filtration onto a cellulose acetate/nitrate membrane in a standard 96-well dot assay apparatus. The reflectances of the precipitation dots were measured by automatic densitometric scanning of the membrane sheets. Standard GAGs produced reflectance values which were related to the number of anionic groups per unit disaccharide; hyaluronate and keratan sulfate

gave lower values while heparin yielded values higher than those of chondroitin sulfates. The presence of 8 M urea, 1% Triton X-100, 30% sucrose, 0.02% $NaN_3$, or mixtures of proteinase inhibitors and various buffers did not markedly influence the reflectances, while 4 M guanidinium chloride and 3 M CsCl reduced the sensitivity of the assay to 30-50 ng. Samples containing sodium dodecyl sulfate (SDS) were not applicable because SDS precipitated with Safranin O. Proteins showed virtually no response, while nucleic acids gave significant although smaller reflectances than GAGs. Owing to its marked sensitivity and convenience the method is particularly suitable for the detection of PGs during their preparative purification and fractionation as well as in various analytical assays.

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     (FILE 'HOME' ENTERED AT 12:56:41 ON 06 JUL 2006)

     FILE 'CAPLUS, MEDLINE' ENTERED AT 12:56:58 ON 06 JUL 2006
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L2            1 S CHONDROITIN (P) PROTEIN? (P) NUCLEIC ACID? (P) ULTRAVIOLET?
L3           12 S CHONDROITIN (P) PROTEIN? (P) NUCLEIC ACID? (P) PURIF?
L4            0 S CHONDROITIN (P) PROTEIN? (P) NUCLEIC ACID? (P) CONTAMINANT?
L5            1 S DERMATAN (P) PROTEIN? (P) NUCLEIC ACID? (P) PURIF?
L6           43 S HEPARIN (P) PROTEIN? (P) NUCLEIC ACID? (P) PURIF?
L7            0 S L6 AND IRRADIA?
L8            3 S L6 AND ULTRAVIOLET?
L9            0 S L6 AND ULTRA-VIOLET?
L10           0 S L6 AND ULTRA VIOLET?
L11           8 S L6 AND MOLECULAR WEIGHT
L12           8 S L6 AND MOLECULAR WEIGHT?
L13          35 S L6 NOT L11
L14          10 S HEPARAN (P) PROTEIN? (P) NUCLEIC ACID? (P) PURIF?
L15           5 S KERATAN? (P) PROTEIN? (P) NUCLEIC ACID? (P) PURIF?
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L5             1 S DERMATAN (P) PROTEIN? (P) NUCLEIC ACID? (P) PURIF?
L6            43 S HEPARIN (P) PROTEIN? (P) NUCLEIC ACID? (P) PURIF?
L7             0 S L6 AND IRRADIA?
L8             3 S L6 AND ULTRAVIOLET?
L9             0 S L6 AND ULTRA-VIOLET?
L10            0 S L6 AND ULTRA VIOLET?
L11            8 S L6 AND MOLECULAR WEIGHT
L12            8 S L6 AND MOLECULAR WEIGHT?
L13           35 S L6 NOT L11
L14           10 S HEPARAN (P) PROTEIN? (P) NUCLEIC ACID? (P) PURIF?
L15            5 S KERATAN? (P) PROTEIN? (P) NUCLEIC ACID? (P) PURIF?
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